Formulation, evaluation and optimization of the felodipine nanosuspension to be used for direct compression to tablet for *in vitro* dissolution enhancement

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Abstract: The oral bioavailability of felodipine very low, nearly just 15% due to its limited solubility and high first pass metabolism. The present study was aimed to improve the rate of the dissolution of Felodipine by formulating a nano suspension of it by combination of high-speed homogenization and media milling technique. Stabilizers screened in this study were Poloxamer 401, HPMC K15M and Tween 80. Concentration of stabilizers were optimized by simplex lattice design for Mean Particle Size (MPS), Poly dispersity Index (PDI), saturation solubility (SS) and *in vitro* drug release in 30 min. The particle size of 201 nm and increase in saturation solubility of nearly 9 folds were obtained for optimize batch. The prepared nano suspension of drug was used as a granulating agent to form tablets having Microcrystalline Cellulose (MCC) as diluents. *In vitro* Drug release study indicates that more than 90% of the drug releases in 30 minutes. Preparing the nano suspension of the low solubility drug is an effective method to increase its saturation solubility. This nano suspension can be prepared effectively by combination of high-speed homogenization and media milling which is also very economical as well

Keyword: High Speed homogenization, Media milling, Simplex Lattice design, Saturation solubility, Process parameter optimization.

INTRODUCTION

The solubility/dissolution behavior is one of the key determinants for the therapeutic effectiveness of a drug and is also rate limiting steps for absorption of drugs from the gastrointestinal tract (Patel *et al*., 2012). Poor solubility of a drug is a major concern for the development of new dosage form because about 10% of the drug in the market, 40% of the drugs in the developing stage and 60% of drugs in synthesis stage have a solubility below 0.1mg/ml (Desai *et al*., 2012) thus it is of the great importance to overcome the low solubility of a drug candidate which is also one of the major obstacle for performing the pharmacological action. According to an estimate 70% of the potential drug candidates never reach to the formulation development stage because of its low bioavailability which is mainly due to their poor solubility in water (Limbachiya *et al*., 2012). According to Biopharmaceutical Classification System (BCS) such drugs belongs to either Class II or IV and their oral delivery often result in low bioavailability, unpredictable absorption and large variations in intra and inter-subject pharmacokinetics (Desai *et al*., 2012).

By using various approaches researchers are trying to shift the drugs from Class II to Class I without changing the pharmacological property. Various formulation techniques are applied to improved the solubility of the drug which leads to improve their dissolution rate and poor therapeutic efficacy (Loftsson *et al*., 2005). The conventional formulation approaches used to tackle this problem are: Making a salt form or complex, using solvent mixture, adding solubilizing agents, incorporating in the drug carrier (e.g. o/w emulsions delivery) but for all these specific approaches the drug need to possess certain physicochemical properties which is the principle limitation of all these formulation approaches for e.g. to make emulsion drug needs to have solubility in oils and to be fit in the Cyclodextrin ring structure drug candidate needs to have right molecular size. The molecular complexation with Cyclodextrins often fail because the high molar ratio of complexing excipient that must be used to in this approach is highly impeding when high dose is to be delivered (Joshi, 2009). Other conventional approaches such as to use excessive amounts of co-solvents to solubilize insoluble drugs poses toxicity problems. This issue is pivotal during drug development when the safety of the agent must be studied in animals, here the dose is quite higher than what is intended for human (Weiner and Bernstein, 1989, SC 2002). One of the such example is the hypersensitivity reaction caused by the Cremophor EL® in Taxol (Gupta and Kompella, 2006).

All these limitations of the specific approaches have shifted the focus of researchers towards the more smarter, the nonspecific approaches which are applicable to almost any drug molecule (apart from a few exceptions) for example particle size reduction. Initially Particle size reduction refers to micronization, which increases results in the increasing the surface area and subsequently the dissolution velocity but does not change the saturation

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solubility so the next consequent step was to go down one further dimension in size and reduces the particle size in nanometers (Thassu *et al*., 2007).

All the benefits that are associated with micro size particles are always there when particle size is reduced to nano level but certain other advantages also accompanies which are exclusively associated with nano size particles such as increased saturation solubility and this method can be used for both compounds which are insoluble in both water and oils. Especially when drug is in crystalline state because it reduces its tendency to get dissolve in any solvent (SH, 1981).

The particle size reduction approach can overcome these delivery issues by obviating the need to dissolve them. It also maintains the drug in a preferred crystalline state and size is also sufficiently small for pharmaceutical acceptability. Nanoparticles are defined as particulate dispersions or solid particles with a size in the range of 10-1000 nm. In most cases drug nanoparticles are suspended in liquid medium (typically water) for better stability and ease of administration which is called "Nanosuspension". Here the pure drug particles might be in crystalline state or amorphous state (Na *et al*., 1999, Mauludin *et al*., 2009, Gao *et al*., 2010). The nanoparticles can be obtained either by particle size reduction of larger particles upto nano level (top-down approach) or by building up particles by precipitation of dissolved molecules (bottom up approach) (Rabinow, 2004).

The precipitation method involves nucleation and the growth of drug particles from dissolved state to the range of nanometer. The primary condition for this is the solubility of the drug in at least one solvent which should be miscible with another non-solvent. Another important parameter is that it should be possible to remove the solvent used in this techniques to an acceptable level in the end products (Gassmann *et al*., 1994, Chen *et al*., 2010) Due to the complexity of process, right now there is no product available in the market based on this technology (Shegokar and Müller, 2010).

Second and more flexible method is the top-down approach by using high-pressure homogenization and media milling. In the formal method, size of the particle reduces by repeatedly forcing a suspension through a very thin gap (typically about 25 μm) at extremely high velocity when the latter method comprises of mechanical attrition of suspended drug particles using milling media such as pearls or balls made of ceramics (cerium- or yttrium-stabilized zirconium dioxide), stainless steel, glass, or highly cross-linked polystyrene resin-coated beads. (Patravale *et al*., 2004, Eerdenbrugh *et al*., 2008). The major limitation for media milling is that usually it takes long time (26-48 hours) for converting drug particles in to the nano stage when the speed is mediocre and the high pressure homogenization requires very costly instrumentations.

Another method is to use high speed homogenizer instead of high pressure homogenizer because it is less costly and easy to operate than high pressure homogenizer but it can hardly give the particle size below 600 nm which will not be sufficient to take full advantage of nano particles. To overcome this limitation in the present investigation we have used combination method (High Speed Homogenization and Media Mill) to prepare the nanosuspension. First pre nanosuspension was prepared by using high speed homogenizer and then this presuspension was media milled in glass vial to produce final suspension.

Felodipine is a BCS class II drug having low solubility (19.17mg/lit) and high permeability. Its low bioavailability (15%) is attributed to both the factors, its low solubility and high first pass metabolism. The present investigation aims to prepare Felodipine nanoparticles to increase its *in vitro* dissolution. As the tablets being most preferred and widely used dosage form, the nanosuspension is used as granulating fluid to prepare tablets using MCC as adsorbent cum filler.

METHODS

Material

Felodipine was obtained as a gift sample from Torrent Pharmaceuticals, Ahmedabad. Poloxamer 407 and Tween 80 were purchased from SD Fine chem, Mumbai, India. HPMC K15 was purchased from ACS Chemicals, Ahmedabad, India. Zirconium oxide beads were obtained as a gift sample from SPARC (Sun Pharma Advanced Research Company), Baroda, India.

Methodology

Preparation of Nanosuspension

The combination approach that includes high speed homogenization and media milling was used to produce nanosuspension. To prepare pre-suspension, drug, surfactant and polymer were mixed in homogenizer cup and it was mixed by using high speed homogenizer. After the completion of homogenization step pre-suspension was transferred to 20ml glass vial containing zirconium oxide beads and was stirred for specific time for the preparation of the final nanosuspension. It was stored at $4^{o}C$.

Optimization of preliminary parameters

In Preliminary study, various preliminary process parameter like, homogenization speed, homogenization time, milling time, concentration of beads, concentration of drug were screened by keeping all the other parameter constant except one which is to be optimized. The parameters were optimized to achieve minimum Mean Particle Size (MPS).

Table 1: Formulation table

Batch Code	Trans formed Value of Component			Actual Value of Component (1%w/vdrug suspension)		
	X_1 Poloxamer407	X_2 Tween80	X_3 HPMC K15	X_1 Poloxamer407	X_2 Tween80	X_3 HPMC K15
F1	1	0	0	1	0	0
F2	0	1	0	0	1	0
F3	0	0	1	0	0	1
F4	0.5	0.5	0	0.5	0.5	0
F5	0	0.5	0.5	0	0.5	0.5
F6	0.5	0	0.5	0.5	0	0.5
F7	0.33	0.33	0.33	0.33	0.33	0.33

Table 2: Mean particlesize (Y_1) and PDI (Y_2), % Drugrelease after 30minand Saturation Solubility of seven different formulations as per simplex lattice design

Batch No.	X1 Poloxamer 407	X2 Tween 80	X3 HPMC K15	Y1 MPS (nm)	Y2 PDI	Zeta potential	% Release after 30 min.	SS
F1	1	0	0	292.9	1.121	9.33	99.85	8.09
F2	0	1	0	197.1	1.265	10.83	99.97	10.9
F3	0	0	1	291.0	0.612	3.40	96.92	7.6
F4	0.5	0.5	0	223.3	0.890	0.84	99.96	8.98
F5	0	0.5	0.5	229.6	0.890	8.08	99.10	8.67
F6	0.5	0	0.5	234.4	1.025	9.00	97.35	7.57
F7	0.33	0.33	0.33	210.4	0.622	17.73	99.97	9

Homogenization speed

Homogenization speed was optimized by changing the speed and keeping times constant and measuring the particle size for each batch.

Homogenization time

Homogenization time was optimized by changing time. Speed was kept constant at speed beyond which more reduction of the particle size was not possible.

Media Milling time

Here the media milling time was selected by keeping speed constant for different time period and finalized on the basis of particle size.

Concentration of beads

Depending on the feasibility of stirring on magnetic stirrer concentration range of beads was selected. The concentrations of beads were considered for screening were 25%, 50%, 75% 100%, 125 % w/v of batch size.

Concentration of drug

The different concentrations of drug tried that were 0.25 %, 0.5%, 0.75%, 1.0% and 1.25% w/v.

Combination method

First the nano suspension was homogenized by using high speed homogenizer then it was subjected to media milling. Here all the parameter were taken from the results of preliminary screening.

Stabilizer optimization

A simplex lattice design was used to optimize the concentration of stabilizer by changing their concentration simultaneously and keeping their total concentration constant. The Mean particle size (MPS) and Poly despesity index (PDI) and saturation solubility (SS), % drug release in 30 min. were selected as response parameters.

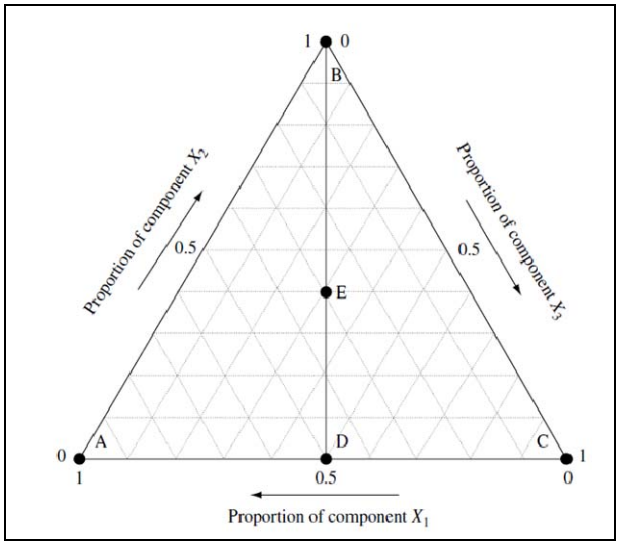


Fig. 1: Simplex lattice design 3 component X_1 X_2 X_3, where $X_1 + X_2 + X_3 = 1$ (X_1= Poloxamer 407, X_2= Tween 80 , X_3 = HPMC K15)

STATISTICAL ANALYSIS

The responses for seven formulations were used to make an equation for simplex lattice design, which can predict

properties of all possible formulation. The regression analysis was performed with the aid of Microsoft excel which gives an idea about the factors that significantly affect the responses.

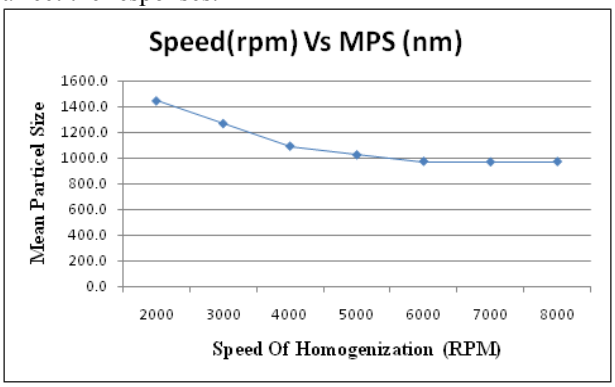


Fig. 2: Optimization of homogenization speed

Evaluation parameter

Particle size, PDI and zeta potential measurement

The average Mean particle size was measured by Zeta analyzer (Zetatrac, MicrotacInc) by diluting the Samples were with distilled water.). It also gives measurement of PDI and zeta potential.

Saturation solubility

Prepared nanosuspension was filled in a vial and kept for 24 hrs stirring to ensure the complete saturation. Samples were then centrifuged, filtered, diluted suitably and analyzed UV spectrophotometer at364 nm.

Shape and morphology

The shape and morphology was examined using Transmission electron microscopy (TEM).

Drug content

The 0.2 ml drug nanosuspension was dissolved in 50ml methanol. The stock solution was sufficiently diluted with methanol and absorbance was measured.

In vitro Drug release study

USP type-2 paddle instrument (ELECTROLAB TDT-06P), (37±0.5˚C, 50 rpm) having Phosphate buffer (pH 6.5) with 1% sodium lauryl sulfate as dissolution medium was used to perform *in vitro* drug release study. Samples of plain drug and spray dried nanosuspension equivalent to 10 mg were added to dissolution apparatus from which at a regular time interval 5 ml sample were taken up to 30 min. which were filtered immediately through 0.1µm PTFE syringe filter (Whatman Inc., Clifton, NJ, USA). Subsequently, 5 ml of fresh medium was added to the dissolution vessel to adjust the volume. Samples were analyzed by UV Spectrophotometer at 364 nm.

Optimization of the formulation

Simplex lattice design was used to optimize the formulation in which the concentrations of Poloxamer407 (X_1), Tween 80 (X_2) and HPMC K 15 (X_3) were chosen as the independent variables. The mean particle size and saturation solubility of nanosuspension were taken as responses (Y), respectively. The equation for simplex lattice model is described as follows:

$Y= \beta_0 + \beta_1X_1 + \beta_2X_2 + \beta_3X_3 + \beta_{12}X_1X_2 + \beta_{13}X_1X_3 + \beta_{23}X_2X_3 + \beta_{123}X_1X_2X_3$ ……. (1)

Table 3: Drug Content (n=3)

Formulation	% drug Content
F1	98.03±0.6
F2	98.85±0.55
F3	98.59±0.39
F4	99.00±0.74
F5	98.9±0.63
F6	99.23±0.25
F7	99.40±0.27

Formulation of the Check batch

Three check point batches were formulated by using the results obtained from the preliminary screening of the formulation parameter.

Selection of the optimized batch

From the checkpoint batch, batch with minimum mean particle size and highest saturation solubility was selected as optimized batch and that was used as granulating agent to make compressed tablet.

Compressed tablet formation

MCC was selected as adsorbent cum diluent to be mixed

Table 4: Results of Regression Analysis

	MPS (Y_1)			*SS (Y_2)*		
	Coefficients	*Standard Error*	*P-value*	*Coefficients*	*Standard Error*	*P-value*
X_1	292.900	0.212	0.000	8.090	0.071	0.006
X_2	197.100	0.212	0.001	10.900	0.071	0.004
X_3	291.000	0.212	0.000	7.600	0.071	0.006
X_1X_2	-86.800	1.039	0.008	-2.060	0.346	0.106
X_1X_3	-57.800	1.039	0.011	-2.320	0.346	0.094
X_2X_3	-230.200	1.039	0.003	-1.100	0.346	0.194
$X_1X_2X_3$	-177.095	6.199	0.022	24.267	2.066	0.054

with the optimized batch of nanosuspension. The granules were prepared by passing it through 40# sieve which were subsequently dried at moderate temperature (60-70˚C). The dried granules were again passed through 40# sieve and retained on 60# sieve. The tablets of 150 mg average weight were prepared on a single station tablet press machine (Cadmach Machines Ltd., India) having concave punches (7.85 mm diameter).

Evaluation of the tablet

Preliminary parameters like bulk density, tapped density, % compressibility, Hausner's ratio, angle of repose were measured for granules. For the final compressed tablets average weight, hardness, % friability and disintegration time were studied.

Table 5: Regression Reduced model

	SS (Y_2)		
	Coefficients	*Standard Error*	*P-value*
X1	8.035705	0.311874	1.64E-06
X2	10.7237	0.311874	3.91E-07
X3	7.519705	0.311874	2.29E-06

Comparative In vitro Dissolution study

The *In vitro* dissolution study of compressed tablets containing nanoparticle was done by the same method as described earlier. Time interval selected for study were 5, 10, 15, 20, 30, 40, 50, 60 Minutes. For comparative study another compressed tablet having same amount of the stabilizer as it is present in nanoparticle was made by replacing nanoparticle with pure drug. In another study just 10 mg drug was directly added in the dissolution media and its release was measured. In this study to simulate the formulation equivalent amount of each stabilizer was added in the dissolution media.

Stability study

Stability studies for tablet were conducted at two different storage conditions for period of 90 days. 1. Room temperature 2. Refrigerated (2-8°C) Three batches were used for each condition.

RESULT

Preliminary study

Homogenization speed

As depicted in the fig. 2 satisfactory results were found at homogenization speed of 6000 rpm which was kept at 3 hrs homogenization time.

Table 6: Process Parameter for Optimized batch

High Speed Homonization	6000 rpm
Time Of Homogenization	3 hour
Beadconc.	100 % (w/w)
Drug Concentration	1 %
Media Milling time	16 hour

Table 7: Composition of Check point batches

Batch	Ingredient Concentration (%)		
	Poloxamer 407	Tween 80	HPMCK15
CH-1	0.3	0.4	0.3
CH-2	0.1	0.7	0.2
CH-3	0.6	0.2	0.2

Results for the effect of Homogenization time indicates that up to 6 hours the particle decreases constantly but after 6 hours no major change in the particle size was observed. So 6 hour was considered the optimum time for 6000 rpm (fig. 3).

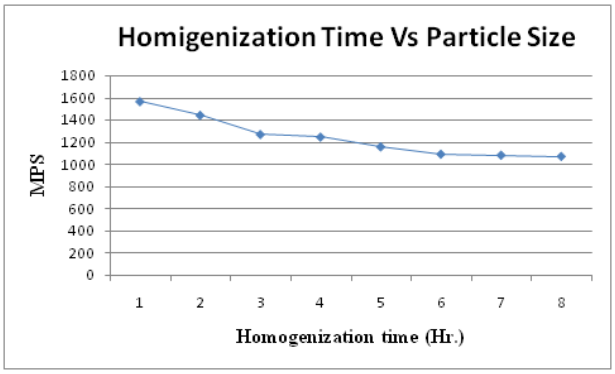


Fig. 3: Effect of Homogenization time on MPS

Optimization of media milling time

The results indicate that as the time increases the MPS decreases due to more attrition up to 36 hours. After 36 hour the more milling have very insignificant effect of the particle size (fig. 4)

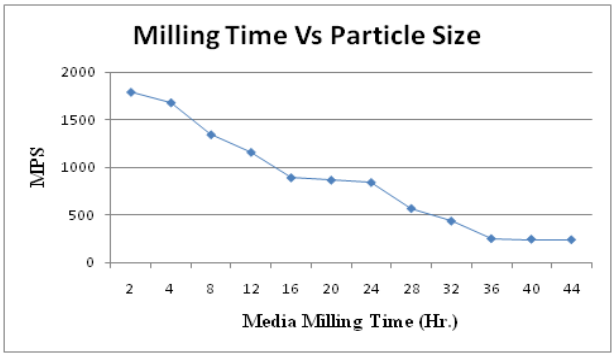


Fig. 4: Optimization of milling time

Concentration of beads

Batch with 100% concentration of beads gave maximum size reduction while maintaining stirring efficiency. (fig. 5).

Concentration of drug

Particle size was decreasing with increase in concentration of drug up to 0.5% w/v concentration. Particle size was nearly constant between 0.5% w/v to 1% w/v. Hence, 1% w/v concentration was chosen for further study looking towards better processing. (fig. 6)

Table 8: Result comparison of predictable value and experimental value

	Y_1(MPS)			Y_2(SS)		
	Predicted Value	Experimental Value	% Deviation	Predicted Value	Experimental Value	% Deviation
CH-1	209.53	238.5	-13.82	8.9	9.0	7.80
CH-2	204.19	201.0	1.56	9.8	9.3	8.85
CH-3	228.73	259.0	-13.23	8.4	8.7	7.34

Effect on Particle size by combining both the methods

The result in fig. 7 implies that by using both the methods in combination time required to get smallest particle size is reduced significantly.

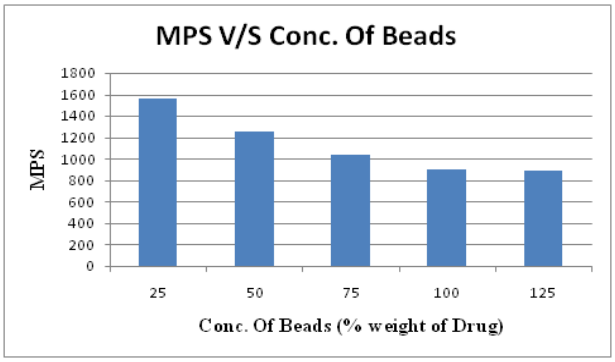


Fig. 5: Optimization of conc. Of beads on MPS

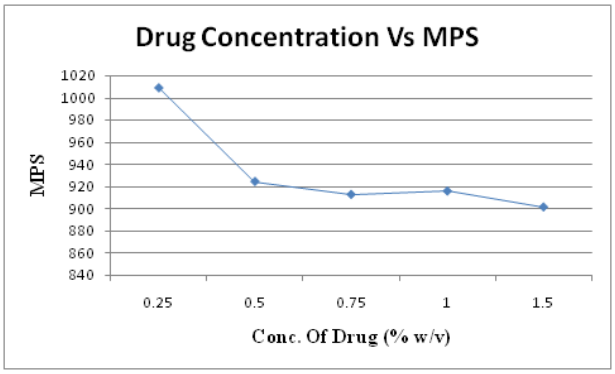


Fig. 6: Optimization of conc. Of Drug

Simplex lattice design

The results of the particle size, PDI and Zeta potential of seven different formulated batches are depicted in table 2. Here fig. 8 gives idea about the particle size distribution for given formulation.

Drug content

Drug content in each formulation was calculated thrice. It was found to be more than 98% in each formulation. (table 3)

In vitro drug release

In vitro Drug release from all the formulation were found to be near to 90 % within 5 min (table 4)

STATISTICAL ANALYSIS

The results of regression analysis performed by using Microsoft Excel 2007 are shown in table 4 and table 5.

Formulation of Check point batch

Table 6 and table 7 describes the process parameter and the formulation parameter respectively used for the formulation of check point batch, which is based on the preliminary study.

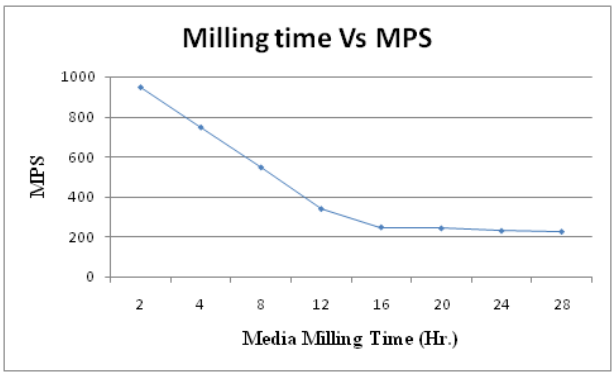


Fig. 7: Optimization of Combine Method

Table 9: Results of Granules evaluation

Evaluation of Felodipineloaded MCC		
Test	Nanoparticles	Drug Powder
Bulk density (g/cm3)	0.38	0.37
Tapped density (g/ cm3)	0.44	0.44
Hausner'sratio	1.15	1.19
Angle of repose	28.81	30.23
Carr's index	13.63	15.90

Table 10: Results of Tablet evaluation

Parameter	Nanoparicles	Drug powder
Diameter (mm)	7.65±0.02	7.63±0.03
Thickness (mm)	1.72±0.01	1.73±0.01
Weight (mg)	150.1±1.9	150.3±1.9
Hardness (Kp)	3.25±0.25	3.00±0.25
% Friability	0.69	0.72
Disintegration time	3.49 Min	12.36 Min

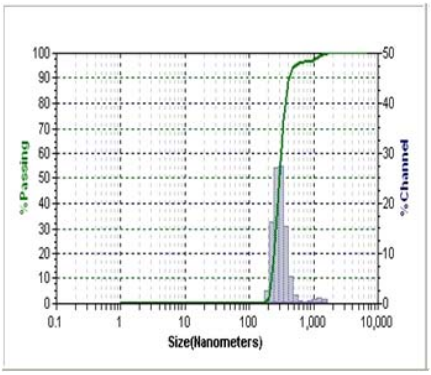
Result comparison for check point batches

As it is evident from the table 8 there is no significant difference between the predicted value and experimental value. This confirms the veracity of the experimental methodology

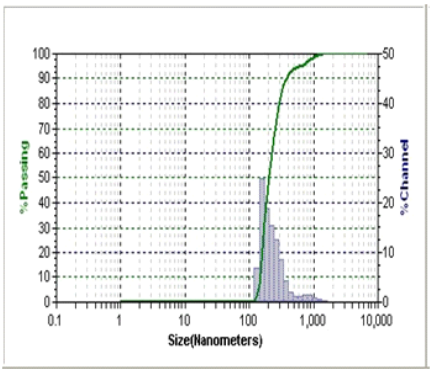
Optimized batch

Based on the results of check point batches, batch CH-2 was found to have minimum MPS and maximum SS

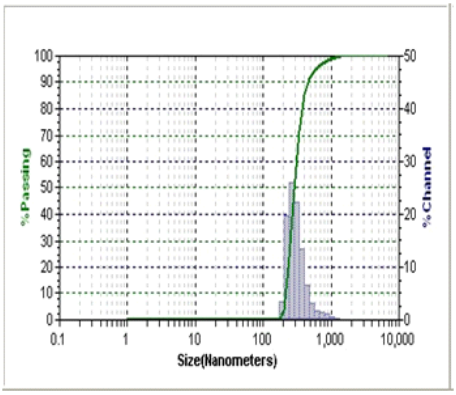
Batch F1



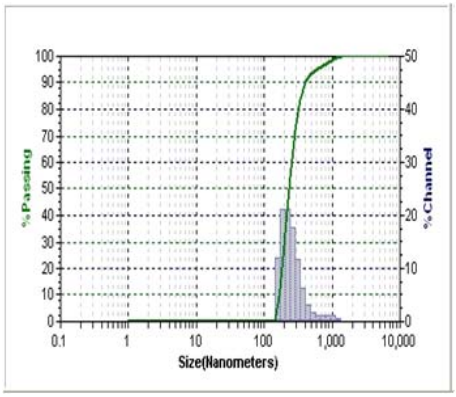
Batch F_2



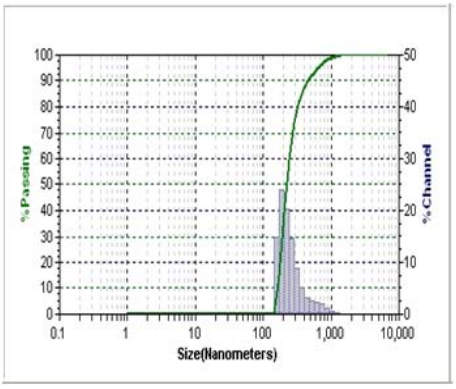
Batch F_3



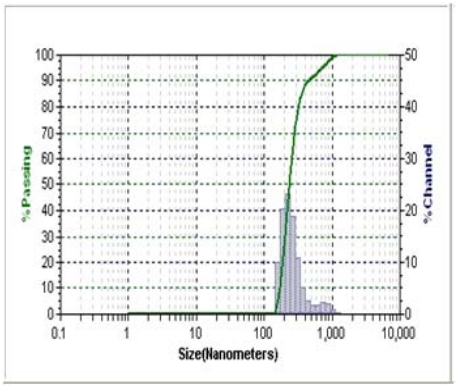
Batch F_4



Batch F_5



Batch F_6



Batch F7

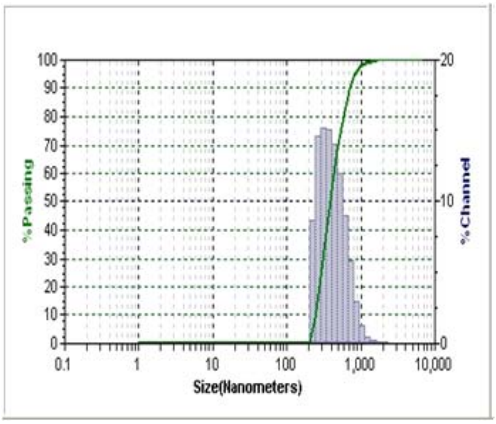


Fig. 8: Particle size distribution graph

hence it was selected as optimized batch to be incorporated in to the tablets and for the further evaluation.

Shape and morphology

The morphological characteristics of the checkpoint batch were observed using TEM (fig. 10). TEM images revealed no aggregation of nanoparticles

Preparation of tablets

Suspension of felodipine nanoparticles and felodipine powder equivalent to 10 mg felodipine was sprayed on MCC using TLC spray gun as uniformly as possible. Equivalent amount of stabilizers were incorporated in the tablets containing felodipine powder for better comparison. Felodipine loaded MCC was then dried in tray drier at 60˚C. The dried MCC was passed through 40# sieve to break any aggregates formed. The tablets of 150 mg average weight were prepared on a rotary tablet press (Minipress, Karnavati Eng. Ltd., India) using 7.85 mm concave punches.

Evaluation of compressed tablet

Table 9 shows the results of granules prepared.

IR-Spectra of the compressed tablet

IR spectra of the compressed tablet containing nanoparticle indicates that all the important functional group are intact (fig. 11). Table 10 shows the evaluation results of granules prepared.

Comparative in vitro dissolution study

Fig. 12 shows the dissolution profiles all three entities (nanoparticle, tablet containing coarse drug powder only).

Stability study

After the storage of 90 days in first 5 min dissolution of the tablet which was kept at room temperature decreases nearly 10% and for freezed tablet to 20% as compared to original one (fig. 13). For tablets kept at room temperature, nearly 90% drug release was achieved in 30 min whereas for the freezed tablets it took nearly 40 min.

DISCUSSION

Here the results of effect of bead concentration indicates that increase in concentration of beads leads to more efficient size reduction that is due to increase in surface area available for milling. The result also implies that by using both the methods in combination (high speed homonization and media milling), time required to nano size the particle is reduced significantly. By using only media milling method 36 hours were required to get a particle size below 300 nm. But if the suspension is pre homogenized then same particle size were achieved in 16 hours. Beyond 16 hours media milling has little effect on particle size.

As depicted in table 2 the Zeta potential value of Felodipine nanosuspension is very low and indicates incipient instability (Le Roy Boehm and Fessi, 2000, Kayes, 1977). This would have provided short time stability in case if only electrostatic stabilization was used but here along with it two polymers HPMC K15 and Poloxamer 407 is also used which can also provide steric stabilization (Thurø Carstensen et al., 1972).

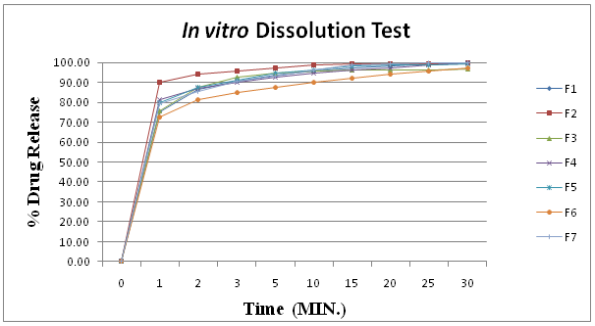


Fig. 9: *In vitro* Dissolution results of F_1 to F_7 batches

So the adsorption layer of steric stabilizer further enhances the stability of the suspension (Verma et al., 2009). This conclusion was further reinforced by the TEM imagines of the check point batch which revealed no aggregation of nanoparticles (fig. 10). In reality the stability of the nanosuspension is indeed higher than being reflected by the measured Zeta potential. During storage the zeta potential remains unchanged. Another factor to be considered is that in this investigation we aimed to use this nanosuspension as granulating agent, to be directly adsorbed on the diluent, to produce granules which can be compressed so the zeta potential is not very significant factor which can affect the stability of final product.

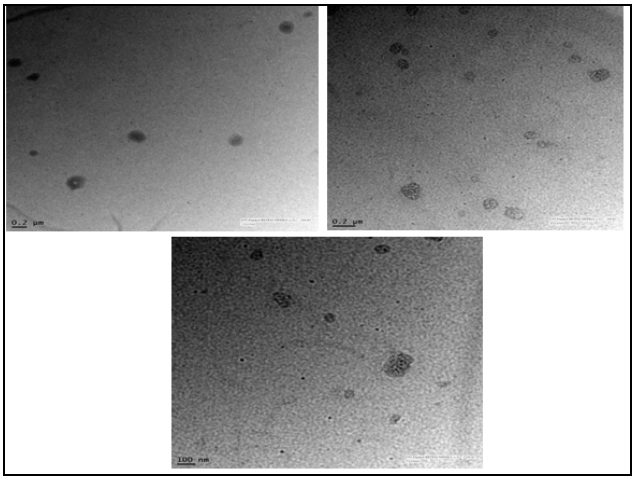


Fig. 10: TEM photograph of Nanosuspension

Statistical Analysis indicates that the major effects (X_1, X_2, and X_3) represent average results of changing one factor at a time from its low to high value, the interactions X_1X_2, X_2X_3, X_1X_3, and $X_1X_2X_3$ show how the responses change when two or three factors change simultaneously.

The results of regression analysis performed by using Microsoft Excel 2007 are shown in table 9.

The result in table 4 indicates that effect of variables X_1, X_2 and X_3 on MPS is positive so increasing any one variable leads to increase in the MPS. Magnitude of effect of X_1 is maximum and that of X_2 is minimum. All first degree interaction terms have negative effects on MPS. Magnitude of effect of X_2X_3 is maximum and that of X_1X_3 is minimum. All the independent variables have significant effect on MPS ($p<0.05$). All the interaction terms have negative values indicating non-additive nature of the effect of independent variables on MPS.

The effects of variable X_1, X_2 and X_3 on SS are positive. Magnitude of effect of X_2 is maximum and that of X_3 is minimum. First degree interaction terms X_1X_2, X_2X_3 and X_1X_3 and overall interaction term, $X_1X_2X_3$, has no significant effect on the response ($p>0.05$) so regression was performed again for SS omitting statistically insignificant terms. The results of the regression analysis are depicted in table 5.

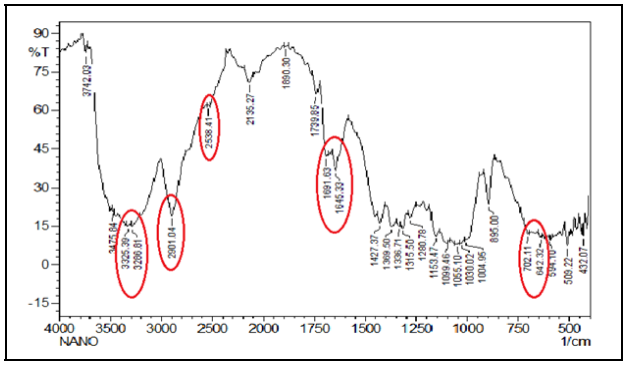


Fig. 11: FTIR of Compressed tablet containing Nanoparticles

Here the results shows that all independent variables have significant effect on the dependent variables ($p<0.05$). The final regression indicates that effect of variables X_1, X_2 and X_3 on SS are positive which implies that increasing any one variable leads to increase in the SS. Magnitude of effect of X_3 is minimum and that of X_2 is maximum.

Paired t–test was performed between full model and reduced model to ascertain that the reduced model efficiently represents the relationship between independent and dependent variables and the difference between full model and reduced model is statistically insignificant. The result of the t-test indicates that $T_{calculated} < T_{critical}$, which implies there is no significant difference between both the models.

While *in vitro* dissolution study when tablet containing nanoparticels were added to media it started to dissolve rapidly, it is due to the high solubility of the nanoparticels, within the first 5 min more than 70% drug was released. Coarse drug containing tablet was not dissolved as readily as of the previous tablet so the % drug release in first 5 min was nearly about 20%. This is due to less solubility of the coarse drug than its nanoparticles and slow release of the drug from the tablet.

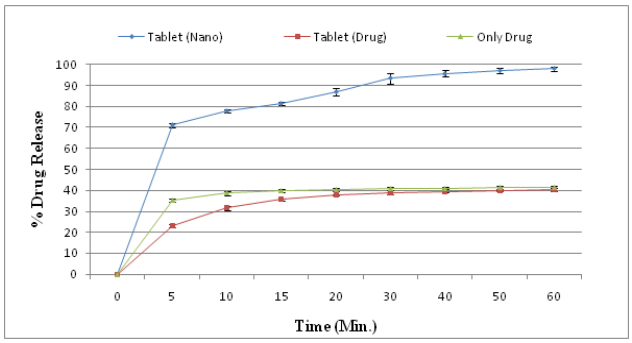


Fig. 12: Comparing *In vitro* dissolution study of table containing Nanoparticle, Tablet containing coarse drug powder and only drug in powder form.

When coarse drug was added in the dissolution media the amount of dissolved drug was below the detectable level. This is obvious because the felodipine is low solubility drug. To increase the dissolution of drug, surfactant Tween 80 equivalent to the concentration in both the tablets was added in dissolution media. After that nearly 35% drug dissolved in the media. After 20 min the percentage drug release from nanoparticle containing tablet and coarse powder containing tablet were nearly 88.0 % and 45.0 % respectively.

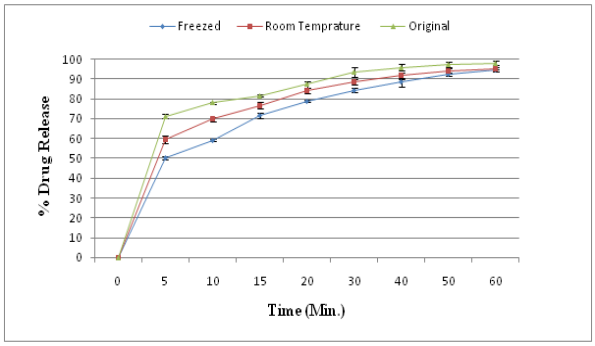


Fig. 13: Comparing *In vitro* dissolution study of table stored at room temperature, store at freezed temperature and original results

After 40 mins more than 90% of the drug got released from the tablet containing nanoparticles where as from the tablet containing coarse powder the release of the drug was almost stagnant. For only coarse powder the stagnant release was found only after 10 min (40%). The obtained results are align with the Noyes–Whitney equation according to which an enlargement of the surface area (i.e. decrease in size) in combination with increased saturation solubility leads to an increase in the dissolution velocity.

The delay in release of the drug from the tablets which were kept for the stability study was due to the increased hardness of the tablets on storage due to the MCC. In both the tablets nearly 95% drug release was achieved in 60 min. This indicates that tablet manufactured by direct compression of nanoparticles are stable in both storage condition and it does not affects its in-vitro release significantly.

CONCLUSION

Felodipine nanoparticles can be produced by combining high speed homogenization and media milling with a size as low as about 200 nm. This method is economical and does not need very costly equipment like high pressure Homogenization. This manufacturing method for nanosuspension was found to be simple and has easy scale up feasibility. By subjecting the suspension to the high-speed homogenization first, time required for media milling can be reduced. This can open the direction of a fast production of nanoparticle of pharmaceutically important drug molecules. Because of being BCS Class-II drug the felodipine nanoparticle exhibits almost 9 folds higher saturation solubility than its coarse powder. The very high release (more than 90% in 30 Min) from tablet will be helpful in developing more such systems for drugs with very low solubility. The reproducibility of the method is confirmed by formulating and evaluating 3 checkpoint batches.

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