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Optimization of felodipine nanosuspensions using Full Factorial Design

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Abstract: The present work is aimed at the optimization of nanosuspension of felodipine, a poorly water soluble antihypertensive drug using Full factorial Design. A 3^2 Full factorial design using Design Expert Version 8.0.7.1was employed to study the effect of the independent variables (diffusing drug concentration and stabilizer concentration) on the dependent variables (particle size, percentage of drug released and polydispersity index). The relationship between the dependent and independent variables was further elucidated using contour plots and response surface plots.The nanosuspensions were prepared by nanoprecipitation with ultrasonnication method using ethanol as solvent and water as antisolvent. The uniform spherical shaped discrete nanoparticles were obtained in the size range of 60 - 300 nm. With increasing drug concentration the particle size decreases while the drug release increases. The uniformity of size indicated by polydispersity index(PI) value was found to be more dependent of stabilizer concentration as with increase in the stabilizer concentration PI decreases showing better homogeneity. The suggested optimized formulation was prepared and the observed values were comparable to the predicted values. It may be concluded that the full factorial design can be used to design and optimize the desired formulation based on different process variables. The optimized nanosuspension showed enhanced release which may lead to enhanced oral bioavailability of felodipine.
Key Words: Nanosuspension, Nanoprecipitation, Felodipine, Factorial Design.

1. Introduction:

Quality by design refers to the achievement of certain predictable quality with desired and predetermined specifications. As different techniques of drug nanosuspensions involve many interacting variables and operating conditions, experimental design methods are extensively being used in the nanosuspension studies. To understand the variables and their interactions, many statistical experimental designs have been recognized as useful techniques. Optimization through experimental design (including factorial design) and response surface methodology is a common practice. [1,2]

Factorial designs are used in experiments where the effects of different factors or conditions on choice for simultaneous determination of the effect of several factors and their interaction. Factorial design is used to study the effect of different variables on the dependent variables of any formulation. Based on the principle of design of experiments, factorial design is employed to investigate the effect of two independent factors. Design of experiments encompasses the use of various types of experimental designs, generation of polynomial equations, and responses over the experimental domain to determine the optimum formulation. Contour plots and response surface plots describe the influence of the independent variables on the selected responses.[3, 4]

Felodipine is, a dihydropyridine calcium antagonist, widely used as a potent antihypertensive drug .[5]It is poorly soluble and oral bioavailability is only 15%. Its dissolution profile is the limiting factor of its bioavailability[6]. In order to increase its dissolution rate several attempts were carried out in the past [7, 8, 9]. Other methods of solubility enhancement like solid dispersions have also been explored for enhancement of solubility of felodipine [10].

Poorly soluble molecules have been successfully formulated by employing a variety of techniques such as: (i) solubilization in surfactant solutions; (ii) use of cosolvents; (iii) pH adjusted solutions;(iv) emulsions; (v) liposomes; (vi) complexation with cyclodextrins; and (vii) solid dispersions[11,12]. However, most of these techniques require a large amount of additives limiting their use from the safety perspective. Moreover, these techniques offer little or no help in the formulation of molecules that are poorly soluble in both aqueous and nonaqueous solvents[13]. Nanosuspensions by the virtue of their large surface area to volume ratio provide an alternative method to formulate poorly soluble compounds. Nanosuspensions are sub-micron colloidal dispersions of discrete particles that have been stabilized using surfactants, polymers or a mixture of both.

The nanoprecipitation technique for nanoparticle manufacture was first developed and patented by Fessi and co-workers [14].This technique presents numerous advantages, in that it is a straight forward technique, rapid and easy to perform. The nanoparticle formation is instantaneous and the entire procedure is carried out in only one step. Briefly, it requires two solvents that are miscible.Ideally, both the polymer and the drug must dissolve in the first one (the solvent), but not in the second system (the non-solvent). Nanoprecipitation occurs by a rapid desolvation of the polymer when the polymer solution is added to the non-solvent. Indeed, as soon as the polymer-containing solvent has diffused into the dispersing medium, the polymer precipitates, involving immediate drug entrapment[15].

The present study, therefore, deals with the optimization of formulation variables to design the best product under conditions of competitive objectives, because interactive effects via a trial-and-error approach are time-consuming and often unsuccessful. Mathematical optimization by means of an experimental design is most helpful in shortening the experimental time[16,17]. The objective of the present work was to apply 3^2 factorial design with desirability function for understanding the quality and optimization of felodipine nanosuspension.A 3^2 factorial design was applied to investigate the combined effect of two formulation variables, the concentration of diffusing drug(X_1) and the amount of stabilizer (X_2) used. The particle size (nm), the polydispersity index and the percentage of drug released after 4 hours were taken as responses (Y). Polynomial equations were used to relate each response to the factors affecting it. Counter plots and response surface plots were drawn and an optimum formulation was selected using the desirability function.

The application of factorial design gave a statistically systematic approach for the formulation and optimization of nanoparticles with desired particle size, polydispersity index and drug release.

2. Materials and methods

2.1. Materials

Felodipine was kindly provided by Glenmark Pharmaceutical Laboratories (Mumbai, India). Ethanol (Rankem, Ranbaxy Fine Chemicals Ltd., RFCL, New Delhi, India) were commercially obtained. Disodium hydrogen phosphate (Merck Specialities Pvt Ltd Mumbai, India), potassium di hydrogen ortho phosphate (Merck Specialities Pvt Ltd Mumbai, India). Sodium di hydrogen phosphate (Merck Specialities Pvt Ltd Mumbai, India), Hydroxy Propyl Methyl Cellulose (Hi-Media Pvt Ltd, Mumbai, India), Poly Vinyl Alcohol (Hi-Media Pvt Ltd, Mumbai, India) were commercially obtained. Deionised water was used for all experiments (Deionizer, MAC, CA-50 5).

2.2. Preparation of nanoparticles

Felodipine nanoparticles were prepared by precipitation–ultrasonnication technique using ethanol as solvent and aqueous medium containing stabiliser as anti solvent (NA). Poly Vinyl alcohol (PVA) or Hydroxy propyl methyl cellulose (HPMC) was used as stabiliser.

2.3. Size measurement and Scanning electron microscopy (SEM)

The particle size and the polydispersity index (PI) was measured immediately after precipitation by dynamic laser light scattering (Zetasizer Ver. 6.11 Malvern Instruments Ltd, UK). The measurement was done in triplicate and size Z-Average (d.nm) and the PI was reported. Particle morphology was observed using scanning electron microscopy (JSM-6360, JEOL Inc., Japan).[18]

2.4 *In vitro* release kinetic experiments

The dialysis membrane technique was used to characterize the *in vitro* release of the prepared nanosuspension using Keshary-Chein glass diffusion cell (donor phase surface area 1.13 cm^2 and receptor phase volume 20 ml). A solvent system of ethanol-water (50:50) was used as receptor medium. The entire system was kept at 37°C with continuous magnetic stirring. The drug released was determined with UV spectrophotometer (UV-1800, Shimadzu, Japan) at 363nm. The results are shown in Fig 2 & 3.

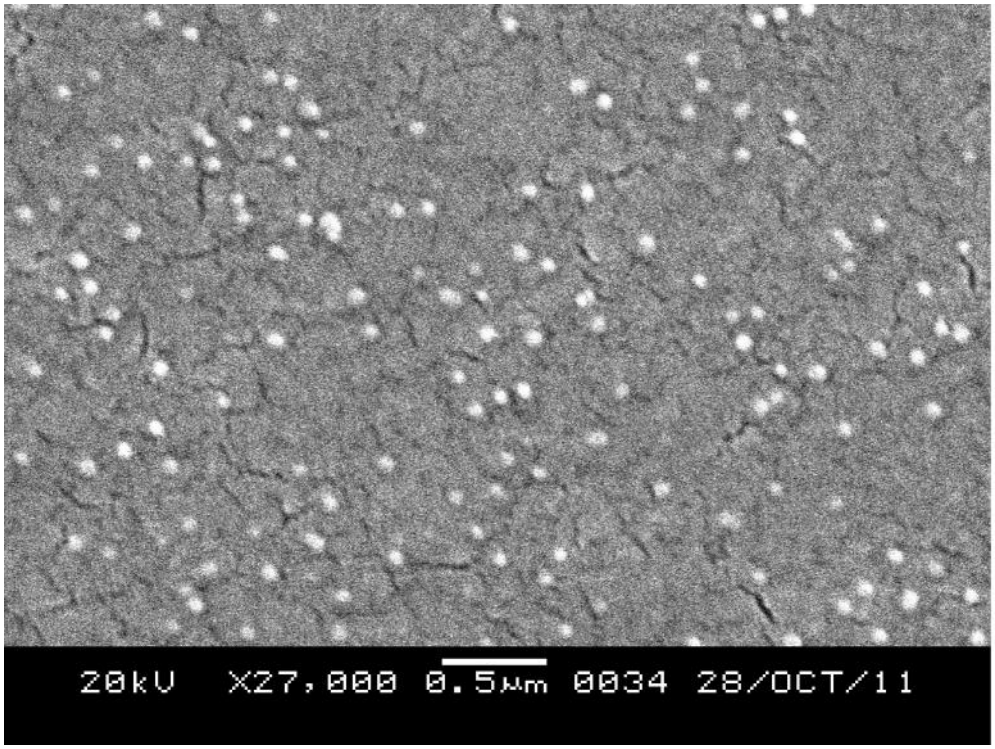

Fig 1: SEM photomicrograph of optimized PVA based felodipine nanopaticles(X 27 000). Scale bar =0.5 µm

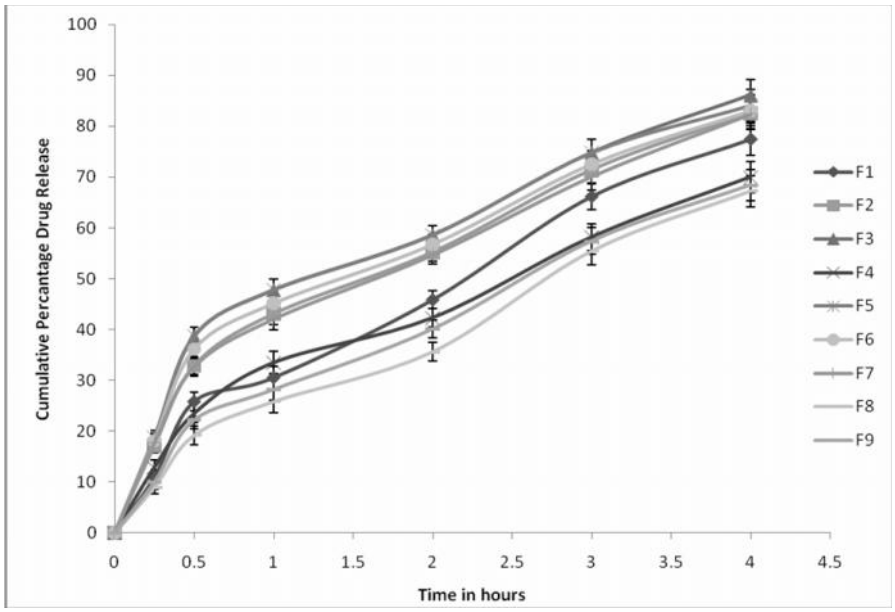

Fig 2: Cumulative percentage of felodipine released from PVA based designed formulations (Each data point represent Mean ± SEM, n=6)

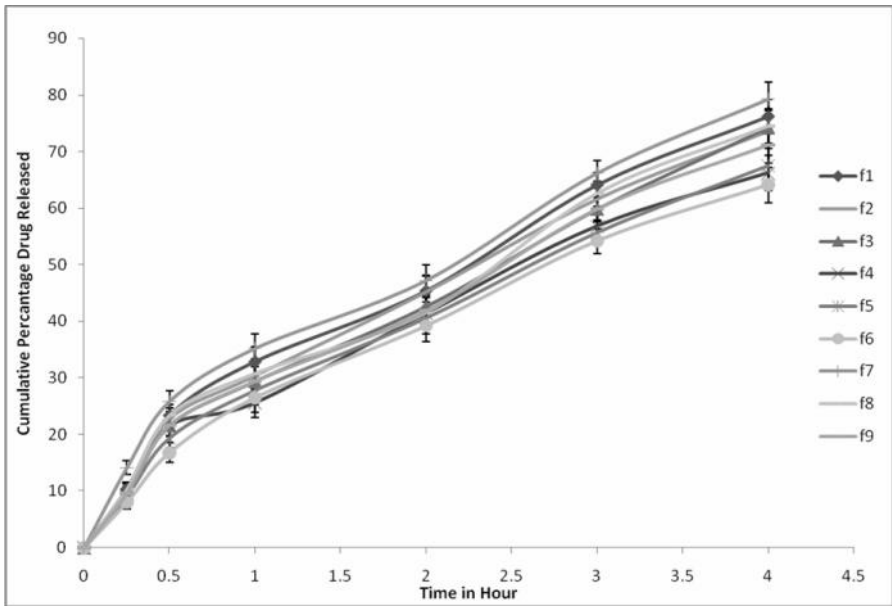

Fig 3: Cumulative percentage of felodipine released from HPMC based designed formulations (Each data point represent Mean ± SEM, n=6)

2.5. Optimization of Formulation using Factorial Design

A Full factorial Design for two factors at three levels each was selected to optimize the response of the variables. The two factors, the concentration of diffusing drug and the amount of stabilizer used were varied, and the factor levels were suitably coded. The particle size (nm), the polydispersity index and the percentage of drug released after 4 hours were taken as the response variables. In this design, two factors are evaluated, each at three levels, and experimental trials were performed for all possible combinations. All other formulation variables and processing variables were kept invariant throughout the study [19].

The effect of the two independent variables diffusing drug concentration (*X*1) and stabiliser concentration (*X*2) on the response (*Y*) was observed. The regression equation for the response was calculated using the following equation-

Response: $Y=b_0+b_1X_1+b_2X_2+b_3X_1^2+b_4X_2^2+b_5X_1X_2$

The responses in the above equation Y are the quantitative effect of the formulation components or independent variables X_1 and X_2; b is the co-efficient of the term X. The main effects (X1 and X2) represent the average result of changing one factor at a time from its low to high value. The interaction term (X1X2) shows how the response changes when two factors are simultaneously changed. The polynomial terms ($X1^2$ and $X2^2$) are included to investigate non-linearity.

2.6. Optimization, data Analysis, and desirability function

Various response surface methodology (RSM) computations for the current optimization study were performed employing Design-Expert software (Version 8.0.7.1, Stat-EaseInc., Minneapolis, MN). Polynomial models including quadratic terms were generated for all the response variables. In addition, 2-D contour plots and 3D graphs were constructed using the output files generated by the Design-Expert software. The significance of these parameters on the variables was assessed by analysis of variance (ANOVA, 2-way).

After fitting of the mathematical model, the desirability function was used for the optimization. During optimization of the formulations, the responses were combined to find a product having the desired characteristics. The desirability function combines all the responses into one variable to predict the optimum levels for the independent variables. A desirability value of 0 represents an unacceptable value for the responses, and a value of 1 represents the most desired value for the responses.

Further, the optimized formulations as selected by the design were prepared and the parameters were observed and compared to the expected values as given by the design. The results are shown in Table 2 and 4.

Table 1: Experimental design and parameters for 3^2 Factorial design for PVA based formulations

Formulation Code	Diffusing drug concen-trations (mg/ml)	PVA (% w/v)	Z Average Diameter (nm) ± S.D	% Release) ± S.D	Polydispersity Index (PI)) ± S.D
F1	40	0.85	191.8± 14.2	77.4 ± 2.1	0.495± 0.065
F2	40	0.15	143.7 ± 21.4	82.6 ± 1.6	0.371 ± 0.024
F3	60	0.75	92.46± 12.1	86.1± 1.4	0.491 ± 0.102
F4	20	0.25	181.6± 16.5	70.0± 2.2	0.571 ± 0.082
F5	68.28	0.50	60.48± 12.8	84.1 ± 2.4	0.466± 0.042
F6	40	0.50	108.5 ± 14.1	83.1± 1.8	0.495 ± 0.035
F7	60	0.25	73.09 ± 12.4	82.4± 1.1	0.453 ± 0.055
F8	11.72	0.50	212.6± 21.4	67.2 ± 1.4	0.750 ± 0.025
F9	20	0.75	171.4 ± 16.2	68.4 ± 1.2	0.510 ± 0.065

Table 2: Experimental and predicted responses for PVA based optimized formulation

Predicted			Observed		
Particle size	PI	% release	Particle size	PI	% release
85.171 ± 21.5	0.54 ± 0.036	85.17 ± 1.8	95.97± 12.5	0.494± 0.026	86.17 ± 1.2

Table 3: Experimental design and parameters for 3^2 Factorial design for HPMC based formulations

Formulation Code	Diffusing drug concentration (mg/ml)	HPMC (%)	Z Average diameter (nm) ± S.D	% Release ± S.D	Polydispersity Index (PI) ± S.D
f1	40	0.85	380.4 ± 31.1	76.2± 1.2	0.443 ±0.012
f2	40	0.15	360.2 ± 26.2	73.6± 1.8	0.411± 0.024
f3	60	0.25	430.4 ± 21.1	74.2 ±2.6	0.661± 0.035
f4	20	0.25	370.6 ± 23.4	66.2 ±2.8	0.332 ±0.045
f5	68.28	0.50	410.7 ± 16.5	67.5 ±2.2	0.668± 0.017
f6	60	0.75	420.2 ± 22.6	64.1± 2.3	0.661± 0.025
f7	40	0.50	310.4 ± 24.2	79.2 ±3.1	0.534± 0.045
f8	11.72	0.50	440.6 ± 15.2	74.5 ±1.9	0.321 ±0.035
f9	20	0.75	370.6 ± 22.4	71.1± 2.1	0.624 ±0.015

Table 4: Experimental and predicted responses for HPMC based optimized formulation

Predicted			Observed		
Particle size	PI	% Release	Particle size	PI	% Release
330.8±21.1	0.529±0.025	78.4±3.1	362.1±18.4	0.624±0.035	76.04±3.4

3. Results and Discussion

3.1. Preparation of nanoparticles

Felodipine nanoparticles were produced by precipitation–ultrasonnication technique. The aqueous phase containing suitable stabilisers (PVA or HPMC) was used as the antisolvent and ethanol was used as solvent. The nanoparticles are formed immediately with rapid precipitation.

3.2. Size measurement and zeta potential analysis

The precipitated drug particles are in the size range of 60 – 300 nm with PVA as stabilizer and 100 – 300 nm for HPMC. The zeta potential of the nanoparticles was found to be negative which may be due to the presence of terminal carboxylic groups of the drug. The results are shown in Table 1 and 3 for PVA and HPMC based nanosuspensions respectively.Scanning electron microscopic studies were carried out for the optimized formulation to observe the physical properties of precipitated nanoparticles. Scanning electron micrograph revealed that the particles were spherical and homogeneous. The particle size as shown in Fig 1 correlated with the results from particle size analysis.

3.3 *In vitro* release studies

With increasing drug concentration drug release increases. The release was higher in PVA based nanosuspensions than HPMC based formulations. The maximum release for HPMC based nanosuspensions was 79% whereas PVA based nanosuspensions showed upto 86.1% in 4 hours. The superior release may be due to the smaller size of nanoparticles.The increase in surface area results in enhanced saturation solubility resulting in superior release.

3.4 Full Factorial Design

Factorial Design for two factors at three levels with -1, 0 and +1 equivalent to a 3^2 factorial design was chosen as the experimental design. This is an effective second-order experimental design associated with a minimum number of experiments to estimate the influence of individual variables (main effects) and their second-order effects. Further, this design has an added advantage of determining the quadratic response surface, not estimable using a factorial design at two levels.[3] To investigate the factors systematically, a full factorial design was employed.

The effect on particle size (Y_1) was observed to be significant by ANOVA and the polynomial equation was found as follows:

$$Y= 108.50-48.05X_1+5.65X_2+13.05X_1^2+19.78X_2^2-0.60X_1X_2$$

The negative sign for coefficient of X1 indicates that as the drug concentration increases, particle size decreases. 3D plots (Fig. 4 and Fig. 5) shows that the particle sizes are towards upper level at low drug concentration and decreases with increase in concentration. Particle size was decreased because increasing drug concentration results in supersaturation, which causes rapid precipitation on diffusion. Therefore, the drug particles were reduced to nanosize ranges, which were efficiently shielded by stabilizer to prevent agglomeration. A smaller concentration of stabilizer induces agglomeration or aggregation and particle size was towards higher level and too much stabilizer promotes Oswald's ripening (a phenomenon in which small crystals, more soluble than large ones, dissolve and re-precipitate onto larger particles). Optimum stabilizer concentration was found between 0.35 to 0.55%.

The effect on % Drug Release (Y_2) was observed to be significant by ANOVA and the polynomial equation was found as follows:

$$Y= 14.60+0.90X_1+0.094X_2-0.75X_1^2-0.42X_2^2+0.83X_1X_2$$

Slow dissolution can be partly attributed to hydrophobicity as evidenced by poor wetting of the drug surface. This causes the particles to aggregate rather than disperse. Dissolution rate in the nanosuspension was improved because of increased surface area. The positive sign for coefficient of X1 indicates that as the drug concentration increases, the % Drug Release (Y_2) also increases. 3D figures as shown in Fig. 4 and Fig. 5 show nearly linear ascending pattern for the values of drug release with decreasing particle size. At higher drug concentration with optimum stabilizer concentration drug release was towards higher level.

Similarly, the effect Polydispersity index (Y_3) was observed to be significant by ANOVA and the polynomial equation was found as follows:

$$Y=0.89+6.652X_1-0.027X_2-0.062X_1^2-0.22X_2-0.033X_1X_2$$

The uniformity of size indicated by PI value was found to be more dependent of stabilizer concentration. The negative sign for coefficient of X2 indicates that as the stabilizer concentration increases, PI decreases showing better homogeneity. Particles were less homogenous at very high drug concentration of stabilizer.

3.5 Formulation optimization using the desirability function

The aim of pharmaceutical formulation optimization is generally to find the levels of the variable that affect the chosen responses and determine the levels of the variable from which a robust product with high quality characteristics may be produced . [2]All the measured responses that may affect the quality of the product were taken into consideration during the optimization procedure. Upon "trading off" different response variables, the following criteria were adopted: particle size ≤ 400 nm and minimized, drug release ≥ 60 % and maximized and PI ≤ 1.0 and minimized. The responses of factorial formulations suggested drug concentration of 60 mg/ml at stabilizer concentration of 0.7 % w/v for PVA based formulations and 40.35 mg/ml and 0.6 % w/v, respectively, for HPMC based formulations as the optimized formulations. The selected optimized formulation was prepared and the observed values were found to be quite comparable to the predicted values.

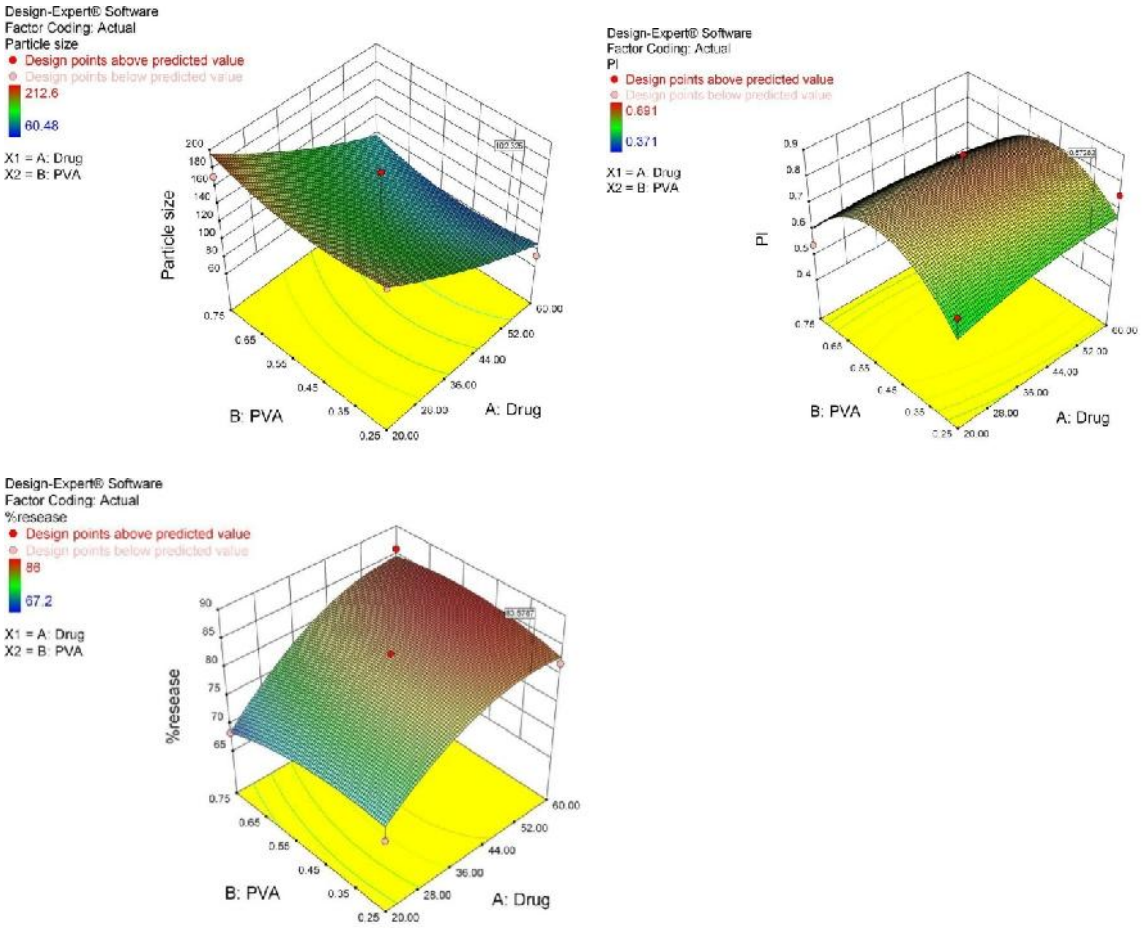

Fig 4: Response surface 3D plots showing effect of independent variables on the dependent variables viz particle size, PI and drug release by design expert on PVA based formulations

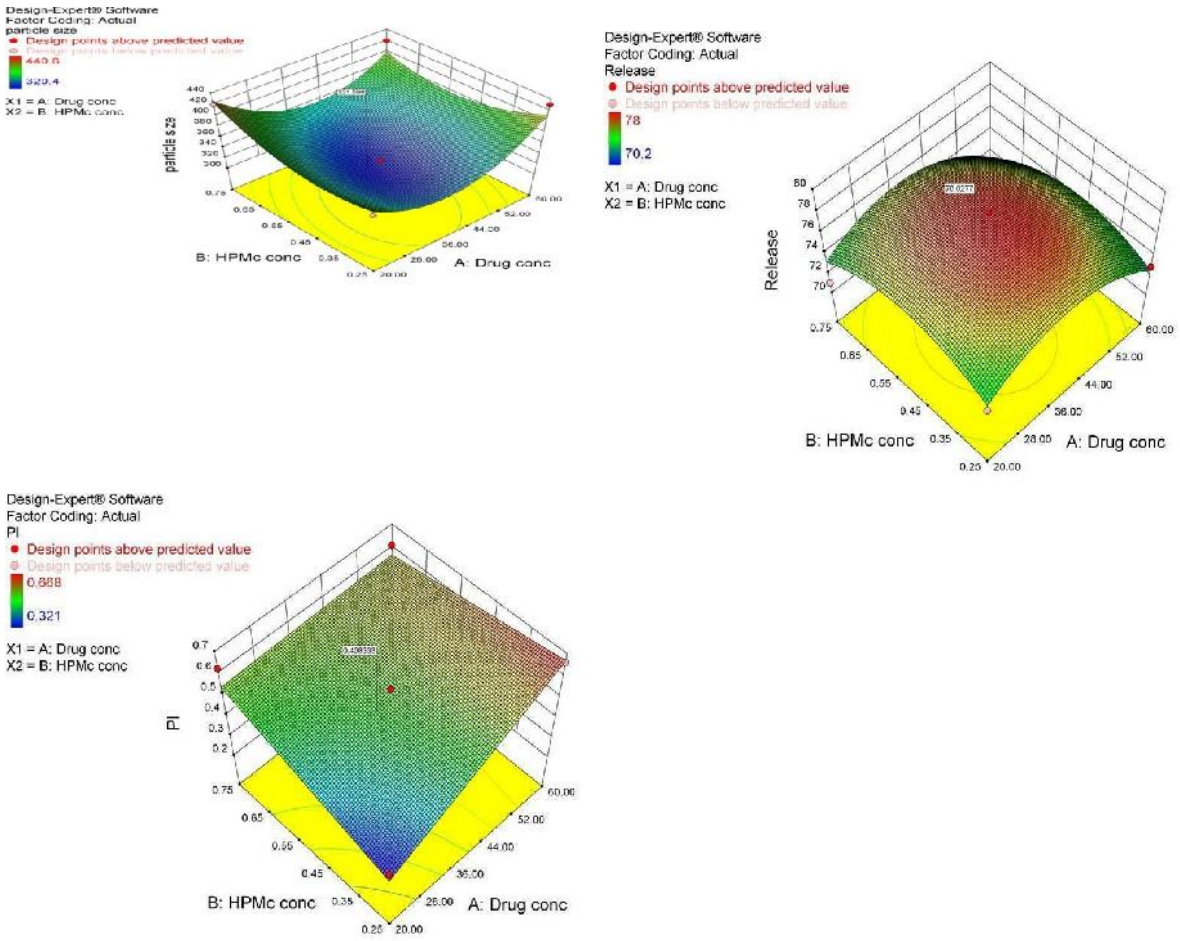

Fig 5: Response surface 3D plots showing effect of independent variables on the dependent variables viz particle size and drug release by design expert on HPMC based formulations

4. Conclusions:

It may be concluded that Factorial Design can be used for a systematic approach for designing and optimizing the desired formulation based on different process variables. With increasing drug concentration the particle size decreases while the drug release increases. The uniformity of size indicated by polydispersity index(PI) value was more dependent of stabilizer concentration as with increase in the stabilizer concentration PI decreases showing better homogeneity. The suggested optimized formulation showed results comparable to the predicted values.The optimized nanosuspension showed enhanced drug release which may lead to enhanced oral bioavailability of felodipine. Since the limited oral bioavailabilty of felodipine is due to its poor dissolution, hence, the increase solubility and thereby the dissolution of felodipine in the form of nanosuspension may enhance the oral bioavailability of felodipine. Further, preclinical trial is warranted to confirm its higher oral bioavailability.

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