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Formulation and Optimization of Nanosuspension Prepared By Media Milling Technique to Enhance the Solubility of Isradipine

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ABSTRACT

Isradipine is a poorly water soluble antihypertensive drug has low bioavailability. The aim of this study was to formulate and characterize isradipine nanosuspension to enhance the solubility of isradipine and thus its bioavailability. Media milling technique was used for the formulation of nanosuspension. The effects of different important process parameters, i.e. the selection of stirring time, selection of concentration of zirconium beads, stirring speed were investigated by preliminary studies while concentration of stabilizers were optimized by simplex lattice design. Concentration of HPMC E3 LV(X1), Carbopol 934P(X2) and PVP K25(X3) were selected as the independent variables whereas mean particle size (Y1), saturation solubility (Y2) and cumulative percentage drug release (cpr) (Y3) were selected as dependent variables. The optimized batch had 100% w/v of zirconium beads, 0.5%w/v of PVP K25 as stabilizer, 0.1%w/v of isradipine, 15 ml of distilled water and 20 hours of stirring time. The particle size and zeta potential of optimized nanosuspension were 248.6 ± 20 nm and 13.96 ± 5 mV respectively. The size of particles of nanosuspension was measured by particle size analyser and transmission electron microscopy (TEM). Differential scanning calorimetry (DSC) and Fourier transform infrared spectroscopy (FT-IR) analysis indicated that there was no interaction between drug and stabilizers. The saturation solubility and *in vitro* dissolution rate of isradipine was significantly increased by particle size reduction and which may leads to increase the bio-availability of the Isaradipine. The stability study of the formulation was carried out for a period of 12 months.

Keywords: Isradipine, media milling, nanosuspension, particle size reduction, saturation solubility, simplex lattice design.

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INTRODUCTION

Isradipine belongs to BCS class II and it is practically insoluble in water (2.28e-01 g/l) and hydrophobicity of isradipine is 2.9. [1] Isradipine has an oral bioavailability of 15-24%. Structure of isradipine is given in figure 1.

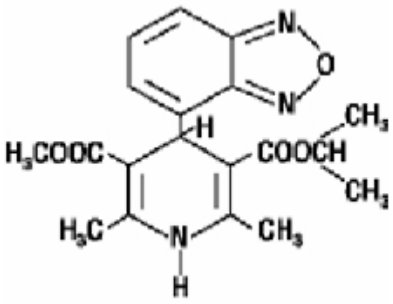

Fig. 1: Structure of Isradipine

Isradipine belongs to the dihydropyridine (DHP) class of calcium channel blockers (CCBs), the most widely used class of CCBs. Isradipine binds to calcium channels with high affinity and specificity and inhibits calcium flux into cardiac and arterial smooth muscle cells. It exhibits greater selectivity towards arterial smooth muscle cells owing to alternative splicing of the alpha-1 subunit of the channel and increased prevalence of inactive channels in smooth muscle cells. Isradipine may be used to treat mild to moderate essential hypertension. Isradipine is available in various dosages 2.5 mg, 5 mg, 10 mg from which 10mg was selected for the all the formulations.

Oral drug delivery system is currently the best standard in the pharmaceutical industries where it is the safest, most convenient and most cost effective method of drug delivery having the highest patient compliance with improved solubility and bioavailability. Solubility is the most important phenomenon of dissolution of solid in liquid phase to give a homogenous system and is one of the most important parameter to achieve desired concentration of drug in systemic circulation. Poorly water-soluble drugs after oral administration often require high doses in order to reach therapeutic plasma concentrations. The bioavailability of an orally administered drug depends on its solubility in aqueous media over different pH ranges. The insufficient dissolution rate of the drug is the limiting factor in the oral bioavailability of poorly water soluble compounds. Various techniques are used for the improvement of the aqueous solubility, dissolution rate, and bioavailability of poorly water soluble drugs include chemical modification, micronization, size reduction (nanosuspension, self-emulsifying system, nanoemulsion) pH adjustment, complexation, solid dispersion, co-solvency, micellar solubilization, hydrotropy etc. [2] About 10% of the present drug molecules are poorly soluble, about 40% of the drug molecules in the pipeline possess a poor solubility, and even 60% of drug molecules coming directly from synthesis have solubility less than 0.1 mg/ml. [3] Thus in this era, when almost all the research-based pharma companies are facing a declining pipeline, the higher proportion of "practically insoluble-in-water" compounds will further reduce the success rate unless there exists an enabling technology to make these drug molecules bioavailable. The problems to find a suitable formulation are even more intense in case of the drugs that are poorly soluble in aqueous media and at the same time in organic media. [4] According to the biopharmaceutical classification, drug molecules with poor water solubility fall either in class II or class IV. Class II drugs show poor solubility, but good permeability, this means that their bioavailability problems can be overcome when improving the solubility while Class IV drugs are characterized by both poor solubility and low permeability.

Thus, nanosuspensions are best suited for poorly water soluble drugs. Nano sized suspension are treated as hopeful means to increase the solubility and thus the bioavailability of poorly water-soluble active ingredients belonging to the classes II and IV in the biopharmaceutical classification system (BCS). [5-6]

A nanosuspension is a submicron colloidal dispersion of drug particles which are stabilized by polymeric stabilizers. A pharmaceutical nanosuspension is defined as very finely dispersed solid drug particles in an aqueous vehicle for oral, topical, parenteral or pulmonary administration. The particle size distribution of the solid particles in nanosuspension is usually less than one micron with an average particle size ranging between 50 to 600 nm. [7]

The principle techniques used in recent years for preparing nanosuspensions can be classified into four basic methods: (A) Wet milling, (B) Homogenization, (C) Emulsification-solvent evaporation, (D) Supercritical fluid method.

MATERIALS AND METHODS

Materials

Isradipine was received as a gift sample from Amneal Pharmaceuticals (Ahmedabad, India) Hydroxy propyl methyl cellulose E3 LV was obtained from Shin-Etsu Chemical Co. Ltd (Tokyo, Japan). Polyvinylpyrrolidone K25 (PVP K25) was acquired from S. D. Fine Chemical Ltd (Mumbai, India). Carbopol 934P was purchased from Lobachemie. Pvt. Ltd (Mumbai, India). Sodium lauryl sulphate (SLS) was purchased from Lobachemie. Pvt. Ltd (Mumbai, India). Yttria-doped Zirconium oxide beads were obtained as a Gift sample from SPARC, India. Polytetrafluoroethylene (PTFE) syringe filters (0.45µm) were purchased from Axiva chem. Biotech (Delhi, India). All other analytical grade chemicals were purchased from SD Fine chemicals (Mumbai, India) and used as received without further alteration. Distilled water was used throughout the study.

Methods of Formulation of Nanosuspension

Media milling technique was adopted for formulation of Isradipine loaded nanosuspension by means of

yttria-doped zirconium beads (SPARC, India) as milling agent. A wide mouth glass vial with outside diameter of 2.5 cm and inside diameter of 2.0 cm, and inside depth of 5.5 cm with total volume 20 mL was used in the milling process. [8] Isradipine was evenly dispersed in an aqueous solution of stabilizer followed by consequent addition of milling agent (zirconium oxide beads). This system was subjected to stirring on magnetic stirrer (Remi Labs, Mumbai, India) for specific period of time. Temperature of this process was maintained at 25-30°C throughout the experiment. The beads were filtered subsequently and Isradipine nanosuspension was stored below 25°C before further studies.

Formulation Optimization by Simplex Lattice Design

The simplex lattice design was adopted to optimize the concentration of stabilizer in nanosuspension. In this design, three factors were evaluated by changing their concentration simultaneously. The simplex lattice design for three component system was represented by an equilateral triangle in figure 2.

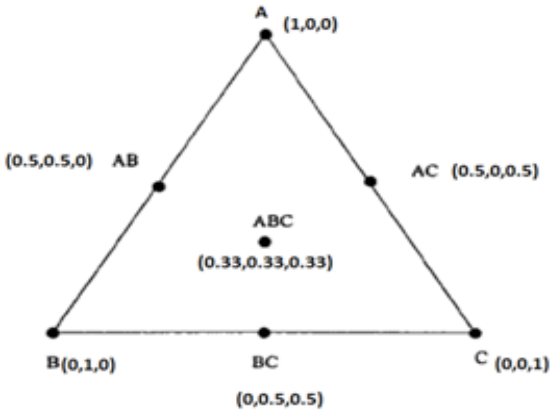

Fig. 2: Equilent triangle of simplex lattice design.

Seven batches of nanosuspension were formulated, including three vertexes (X_1,X_2,X_3), three half way points between vertices (X_1X_2,X_2X_3,X_3X_1) and one centre point (X_1,X_2,X_3). Code representation of formulation with actual and transformed values was shown in Table **1**. The concentration of stabilizers like HPMC E3 LV (X_1), Carbopol 934P (X_2), and PVP K25 (X 3) were selected as independent variables. Mean particle size (Y_1), saturation solubility (Y_2) and cumulative percentage drug release after 10 min (Y_3) were taken as dependent responses. Actual values of all independent variables are given in table **2**, whereas actual values of all the ingredients present in the formulation are given in table **3**. The responses for seven formulations were used to fit an equation for simplex lattice design which could predict properties of all possible formulation. Regression analysis was employed to determine the control factors that significantly affect the responses. [9]

Multiple Regression Analysis

Simplex lattice design was adopted to optimize the formulation of nanosuspension containing isradipine. The concentrations of HPMC E3 LV, Carbopol 934P, and PVP K25 were chosen as independent variables. The mean particle size, saturation solubility and drug release after 10 min from nanosuspension were taken as dependent variables. The general equation 1 for simplex lattice model is described as follows:

$$Y= \beta1X1 + \beta2X2 + \beta3X3 + \beta12X1X2 + \beta13X1X3 + \beta23X2X3 + \beta123X1X2X3 \quad (1)$$

According to simplex lattice design and the selected concentration ranges of HPMC E3LV, Carbopol 934P, PVP K25, and seven different formulations of nanosuspension containing isradipine were constructed.

Table 1: Code representation of different formulation.

Formulation code	Code represent	Concentration transformed value		
		HPMC E3 LV	Carbopol 934P	PVP K25
F1	X_1	1	0	0
F2	X_2	0	1	0
F3	X_3	0	0	1
F4	X_1X_2	0.5	0.5	0
F5	X_1X_3	0.5	0	0.5
F6	X_2X_3	0	0.5	0.5
F7	$X_1X_2X_3$	0.33	0.33	0.33

Table 2: Actual value of X_1, X_2 and X_3.

Variables	Lower Level (0) (%w/v)	Higher Level (1) (%w/v)
X1(HPMC E3 LV)	0	0.5
X2 (Carbopol 934 P)	0	0.2
X3 (PVP K25)	0	0.5

Table 3: Actual values of all the components present in the formulation.

Ingredients	F1	F2	F3	F4	F5	F6	F7
Drug(mg)	15	15	15	15	15	15	15
HPMC E3 LV(mg)	75	0	0	37.5	37.5	0	24.75
Carbopol 934P(mg)	0	30	0	15	0	15	9
PVP K25(mg)	0	0	75	0	37.5	37.5	24.75
Zirconium beads (g)	15	15	15	15	15	15	15
Water(ml)	15	15	15	15	15	15	15
Stirring time (hours)	20	20	20	20	20	20	20

Characterization

Particle Size

Mean particle size of nanosuspension was perceived by laser diffraction technique using particle size analyzer (Microtrac, Mumbai, India). 1 mL sample of isradipine nanosuspension (1mg/mL) was diluted with 10 mL of deionized water followed by ultra-sonication (typically for 20–30 s) prior to size analysis to disperse any aggregates. The mean particle size was calculated based on the particle size values of three independent measurements.

Zeta Potential

The zeta potential specifies physical stability of colloidal systems and revealed extent of electric charge at the surface of the particles. Particle electrophoretic mobility was exploited for zeta potential values using Microtrac (Mumbai, India). All the measurements were accomplished using deionized water.

Saturation Solubility

Isradipine loaded nanosuspension (5 mL) was subjected to centrifugation at 15000 rpm for 60 minutes (Remi Labs, Mumbai, India). The supernatant was examined for drug content by UV-visible spectrophotometer (UV-1800, Shimadzu Corporation, Tokyo, Japan) at 331 nm. All samples were analyzed in triplicate. [10]

Dissolution Study

Dissolution was accomplished at 37±1°C in USP type II (paddle) apparatus at speed of 100 rpm using 0.1 M HCl+ 2% SLS. [11] Nanosuspension was directly introduced to the dissolution media and aliquots were withdrawn at predetermined time intervals and instantaneously filtered through 0.45µm PTFE (polytetrafluoroethylene) syringe filter (Axiva, Delhi, India) to curtail the drug adsorption and precipitation. [12] All the samples were analyzed for drug content by UV- visible spectroscopy method as mentioned previously at 331 nm. [11] For contrast pure drug powder of isradipine was also used.

Transmission Electron Microscopy (TEM)

The optimized batch of isradipine loaded nanosuspension was subjected for 10–15 min on a coated carbon grid stained with 2% uranyl acetate solution subsequently washed with distilled water. Isradipine nanosuspension particles were observed by TEM (Tecnai T20, Philips, Holland). Radiation generated at 200 kV as X-ray source with camera length of 100 cm was utilized. Two dimensions of X-ray patterns were photographed by the camera.

Differential Scanning Calorimetry (DSC)

Thermal studies of isradipine, HPMC E3 LV, Carbopol 934P, PVP K25 and physical mixture was performed using Differential Scanning Calorimeter (DSC 60, Shimadzu, Japan). The samples were hermetically sealed in an aluminum crucible before analysis. The system was purged with nitrogen gas at a flow rate of 100 mL/min. Heating was done between 30°C to 250°C at rate of 10°C/min. [13]

Fourier Transform Infrared Spectroscopy (FT-IR)

The infrared spectra of the samples were obtained using an IFS-55 Fourier transform infrared spectrometry (FT-IR) spectrometer (Thermo scientific, Japan). The measurements were performed over the range of 400–4,000 cm^{-1}. Powder samples were milled with KBr to form a very fine powder. This powder was then compressed into a thin pellet for analysis and their IR spectra were recorded where as for the liquid sample direct analysis was carried out directly over the region 400 to 4000 cm^{-1} in the instrument.

Stability Study

Isradipine loaded nanosuspension was subjected to stability studies as per International Conference on Harmonization (ICH guidelines). Sample was kept in siliconized glass vials at two different conditions; long term testing 25 ± 2°C/ 60% RH ± 5% for 12 months and accelerated testing 40 ± 2°C/75% RH ± 5% for 6 months. At different pre-set time intervals, samples were examined for variation in their mean particle size, drug content and cumulative drug release after 90 min

Table 4: Preliminary trials.

Formulation Parameter	levels	Mean particle size*
Stirring time	10 hours	2955 ± 26 nm
	15 hours	645 ± 30 nm
	20 hours	474 ± 32 nm
Stirring speed	800 rpm	1144 ± 31 nm
	1100 rpm	1354 ± 23 nm
	1400 rpm	474 ± 32 nm
Concentration of zirconium oxide beads	50% w/v	934 ± 29 nm
	100%w/v	474 ± 32 nm
	150% w/v	924 ± 31 nm

*Results are in mean ± S.D (n=3)

RESULTS AND DISCUSSION

Media milling technique contains a milling chamber filled with the milling media, water, drug and stabilizer. Media milling have many advantages over other methods like no use of harmful organic solvents, high stability of formulation, ease of scale-up, and narrow size distribution of particles in the nanosuspension, little batch-to-batch variation. Hence, media milling technique was adopted as simple, economical for formulation of isradipine loaded nanosuspension. The milling medium contains zirconium oxide beads. The energy input to break the micro particulate drug into nano-sized particles was delivered by high energy shear forces generated as a result of the impaction of the milling media with the drug particles. Preliminary trials were performed to decide various processing parameters. All batches of preliminary trials were checked for particle size since the major aim of the work was to reduce particle size of isradipine. As depicted in Table 4, the result shows that the stirring time of 20 hours was found to be optimum for size reduction of the particles.

Moreover, stirring speed of 1400 RPM was optimized (Table 4). Similarly, for the concentration of zirconium beads 100% w/v was the optimum concentration for the formulation of nanosuspension (Table 4).

Effect of Stabilizers on Stability of Nanosuspension

Stability is the major factor which affects the nanosuspension thus it was major challenge to deal. Ideally there are two types of stabilizers which provide stability to the nanosuspension one is ionic stabilizers and the other is nonionic polymeric stabilizers. Concentration and the type of stabilizer have major effect on the stability of nanosuspension. Nonionic Polymeric stabilizers cover full surface of the particles and gives stability whereas ionic stabilizers stabilizes the system by forming ionic barriers. Isradipine is a non-polar molecule which fails to have affinity with ionic stabilizers so, nonionic polymeric stabilizers like HPMC E3 LV, Carbopol 934P, and PVP K25 were selected for the optimization of nanosuspension formulation.

Experimental Design

During the formulation of various pharmaceutical dosage forms, numerous formulation and process

variables involving cost effectiveness and usefulness should be optimized correctly. Factorial design is used often to optimize the formulation variables. Factors like concentration of HPMC E3 LV(X1), Carbopol 934P(X2) and PVP K25(X3) have significant influence on mean particle size (Y1), saturation solubility (Y2) and cumulative drug release (CPR) after 10 min (Y3) of isradipine loaded nanosuspension. Hence, they were utilized for further analysis. For all 7 batches, all the three selected dependent variables (Y1, Y2, and Y3) showed a wide variation in mean particle size, saturation solubility, CPR after 10 minutes. The responses of all dependent variables of simplex lattice design are shown in table 5.

The polynomial equations can be used to predict conclusions after considering magnitude of coefficients and mathematical sign which is either positive or negative. Regression analysis coefficient results of dependent variables are given in table 6.

Effect of Formulation Composition Factor on Particle Size

The major aim of the present research was to increase dissolution velocity and solubility of poorly water soluble drug; Isradipine. Counter plot for X1 (Fig. 3) illustrated strong effect of three factors (concentration of HPMC E3 LV, Carbopol 934P, PVP K25).

Table 5: Responses of dependent variables for all batches of simplex lattice design.

Batches	Mean particle size(nm) ± S.D*	Saturation solubility (µg/ml) ± S.D*	Cpr within 10 minutes ± S.D*
Pure drug	2955 ± 20	12.72 ± 3	14.08 ±0.04
F1	327 ± 13	92.76 ± 10	62.86 ±0.03
F2	290.3 ± 10	36.00 ± 2	67.94 ±0.06
F3	248.6 ± 20	56.00 ± 4	70.20 ±0.2
F4	322 ± 8	90.96 ± 15	66.84 ±0.1
F5	294.4 ± 6	67.76 ± 20	67.57 ±1
F6	260.3 ± 15	27.88 ± 5	68.71 ±0.2
F7	335 ± 10	73.44 ± 6	66.10 ±1

*Results are in mean ± S.D (n=3)

Table 6: Regression analysis results of dependent variables of nanosuspension.

Factors	Mean particle size coefficient	Saturation solubility coefficient	CPR in 10 min. coefficient
X1	327(β1)	92.7(β1)	62.86(β1)
X2	290(β2)	36(β2)	67.94(β2)
X3	248.6(β3)	56(β3)	70.2(β3)

A lowest particle size of 248.6±20 nm was observed with concentration of PVP K25 0.5%w/v (Batch F3). This might be because of stearic stabilization and prevention of Oswald ripening by PVP K25.

Effect of Formulation Composition Factor on Saturation Solubility

The second response, saturation solubility was also had strongly influenced by all three independent variables (Fig. 4). Highest saturation solubility value (92.76±10µg/mL) was observed with Batch F1. It indicates increase in saturation solubility of isradipine with increase in the amount of HPMC E3 LV. It is because of the solubility of HPMC E3 LV is better than Carbopol 934P and PVP K25 in water. The saturation solubility of optimized batch (F3) is lower compared to F1 batch due to the lower solubility of PVP K25 in water.

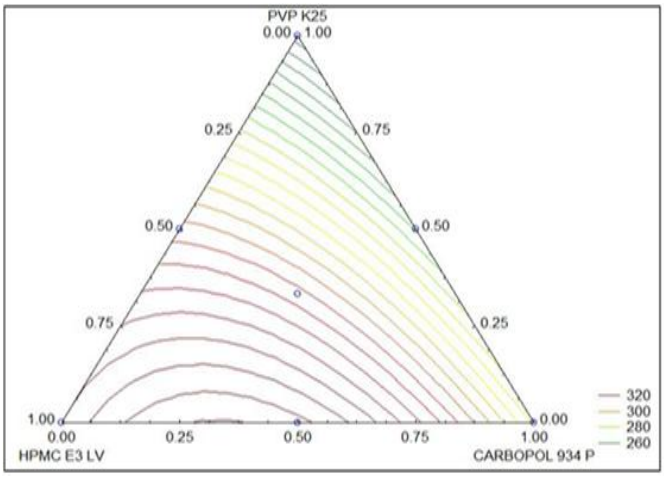

Fig. 3: Counter plot for mean particle size.

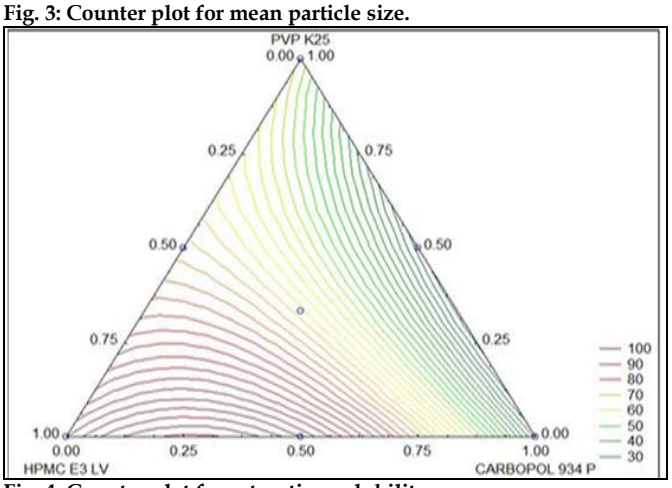

Fig. 4: Counter plot for saturation solubility.

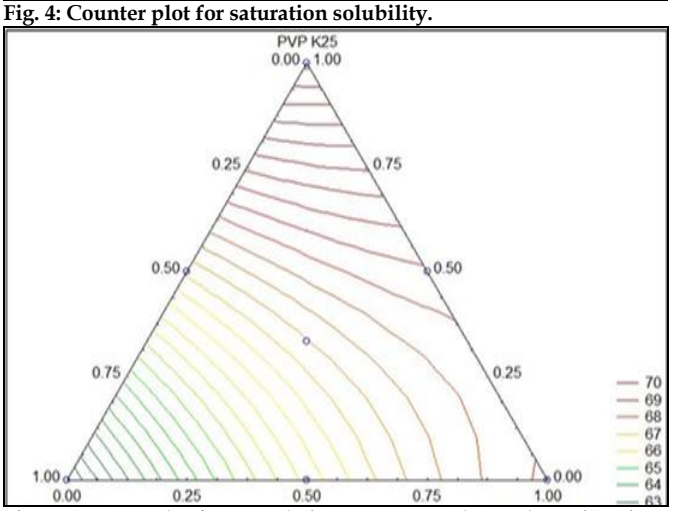

Fig. 5: Counter plot for cumulative percentage drug release (CPR).

Effect of Formulation Composition Factor on Cumulative Percentage Drug release (CPR) after 10 Minutes

The third response, CPR was also affected by all the independent parameters. Highest CPR after 10 min (70.20±0.2%) was observed with batch F3. It indicates the increase in dissolution rate with decrease in particle size (Fig. 5).

The particle size of F3 batch was found lowest among all other batches so, it was concluded that the dissolution rate of F3 batch is highest among others due to lowest particle size. Overlay of all three dependent variables is given in figure 6. From, which the common region for all three variables is given in dark border.

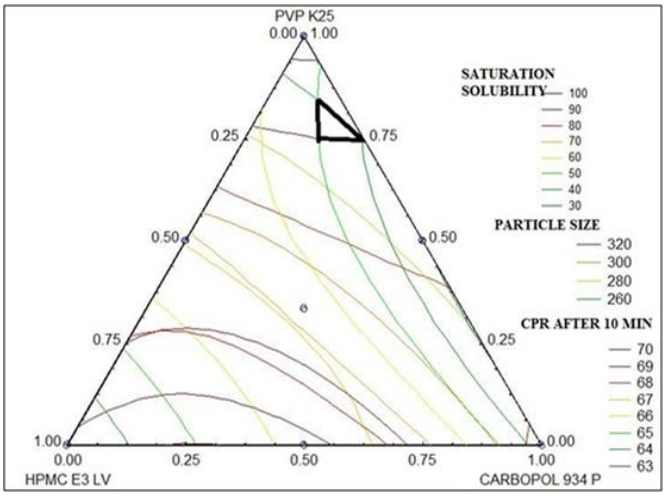

Fig. 6: Overlay counter plot for all dependent variables.

Table 7: Responses of checkpoint batch

Response	Experimental*	Calculated value	% Relative error
Y1 (Particle size)	322 ± 22nm	309.2 nm	4.13
Y2 (Saturation solubility)	67.20 ± 21µg/ml	70.18µg/ml	4.43
Y3(cpr after 10 minutes)	60.37 ± 1.3%	63.54 %	5.25

*Results are in mean ± S.D (n=3)

Checkpoint Batch Analysis

Check point batch of nanosuspension was prepared experimentally using the same procedure. The result of particle size, saturation solubility, and CPR after 10 minutes values was compared with that of computed values from the regression equations. When both (experimentally obtained and theoretically computed) mean particle size, saturation solubility, cpr after 10 minutes values were compared and there was no significant difference found between them (Table 7).

Characterization

Particle Size

Mean particle size affects saturation solubility, dissolution velocity, physical stability of nanosuspension. [14] There is an overall tendency of particles in the dispersed systems to aggregate and grow. This phenomenon is known as Ostwald ripening. Due to higher saturation solubility of the small particles, the solute concentration is higher in the vicinity of the smaller particles than that of the larger ones. So the solutes will diffuse from the surface of the small particles to the large particles due to the concentration gradient, and aggregate on the surface of the larger particles. The continual dissolution of the small particles and aggregation of the solute on the surface of the large particles led to the formulation of micro particles. However, the narrow size distribution conduced to the absence of the Ostwald ripening by eliminating the different solute concentration among the medium. So the non-uniformity of the particle size is another factor influencing stability of nanosuspension besides the high surface energy. Particle size distribution of one of the optimized batch is shown in Figure 7.

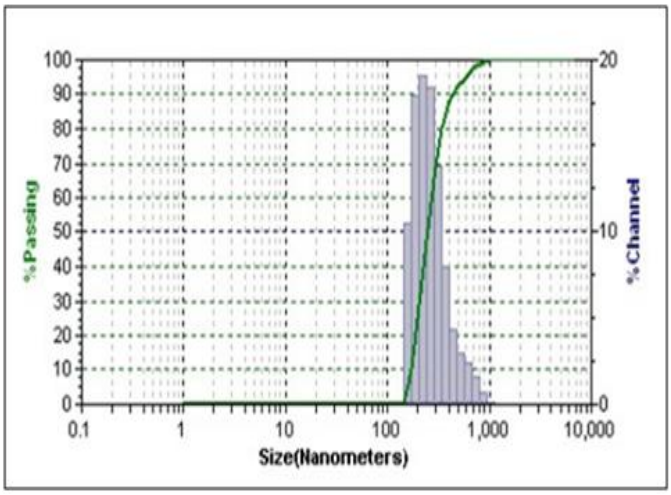

Fig. 7: Particle size distribution of optimized batch of Isradipine nanosuspension.

Mean particle size of optimized batch was 248.6±20 nm.

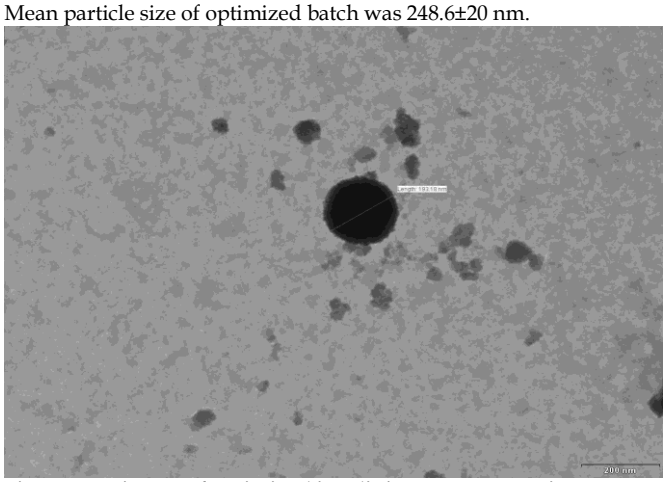

Fig. 8: TEM image of optimized isradipine nanosuspension.

Zeta Potential

Zeta potential is used to check the long-term physical stability of nanosuspension which is based on measurement of movement of the particles in an electric field. The particles which have enough zeta potential to provide appropriate electric repulsion or steric barriers to provide sufficient steric repulsion between each other. A zeta potential of at least ± 30 mV for electrostatically and ± 20 mV for sterically stabilized systems is desired to achieve a physically stable nanosuspension. [15] Zeta potential of optimized isradipine loaded nanosuspension batch was found to be 13.96 ± 5 mV which is sufficient for stability.

Saturation Solubility

The determination of the saturation solubility is very important because it affects bioavailability of the drug. Saturation solubility of optimized batch (F3) of nanosuspension and bulk drug was 56.00 ± 4µg/mL

and 12.72 ± 3µg/mL, respectively which was 4.4 times higher than that of bulk drug.

Transmission Electron Microscopy (TEM)

The morphology of the nanosuspension was checked using TEM (Fig. 8).

TEM images revealed no aggregation of nanoparticles. It was also observed that nanoparticles were of nonuniform size and of spherical shape.

Dissolution Study

The dissolution profiles of pure drug and nanosuspension are shown in figure 9.

The formulated nanosuspension released about 70.20 ± 0.2% of drug within 10 minutes and 99.5 ± 0.5% of drug in 90 minutes (F3 batch) whereas the pure drug released only 14.08 ± 0.04% within 10 minutes and 31.66 ± 0.2% within 90 minutes. So, it was concluded that the particle size reduction increases the dissolution rate and formulation of nanosuspension for poorly water soluble drugs has dramatic effect on dissolution rate and thus on bioavailability. From the Nernst-Brunner and Levich equation (Eq. 2), we can say that the increased surface area (A) and saturation solubility (CS) due to the decreased particle size led to the increased dissolution velocity:

$$\frac{dx}{dt} = \frac{DA}{h} * \left(Cs - \frac{X}{V}\right) \quad \ldots\ldots (2)$$

dX/dt is the dissolution rate, D is the diffusion coefficient, A is surface area of particle, h is the diffusional distance, CS is saturation solubility of drug, X is concentration in the surrounding liquid, and V is volume of the dissolution medium.

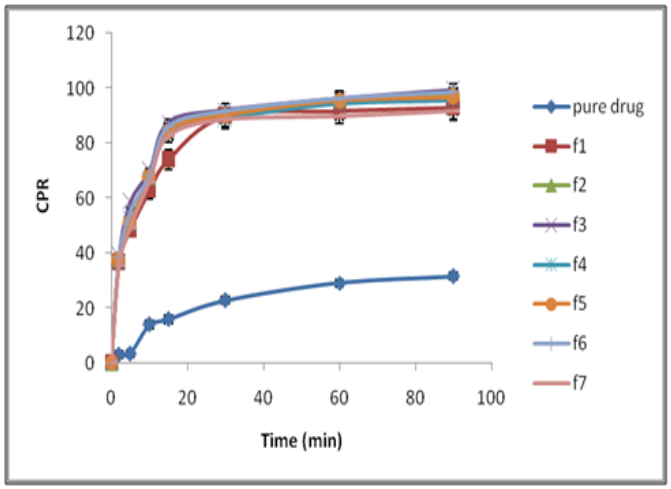

Fig. 9: Comparison of dissolution profile of all batches of simplex lattice design with pure drug.

Fourier Transform infrared spectroscopy (FT-IR)

FTIR analysis was used to evaluate the possible intermolecular interactions between isradipine and the excipients (stabilizers). The spectra of pure isradipine, HPMC E3 LV [18], Carbopol 934P [19], PVP K25 [16], physical mixture and nanosuspension of optimized formulation are shown in Figure 10.

It was found that there was no interaction occurred in the final formulation between stabilizers and drug. The peaks of various groups like N-H stretching, C=O stretching, C-H stretching remains constant in the physical mixture as well as in the optimized formulation and there is no shifting of any peak of pure drug. So, we can conclude that there might not be any interaction occurred between the drug and stabilizers.

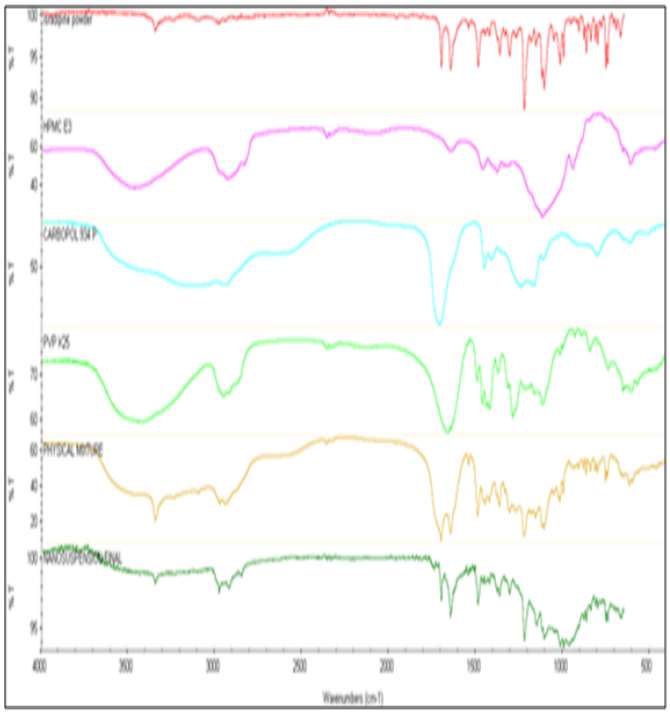

Fig. 10: Comparision of FT-IR spectra of isradipine pure drug powder, HPMC E3 LV, Carbopol 934P, PVP K25 with physical mixture and optimized nanosuspension.

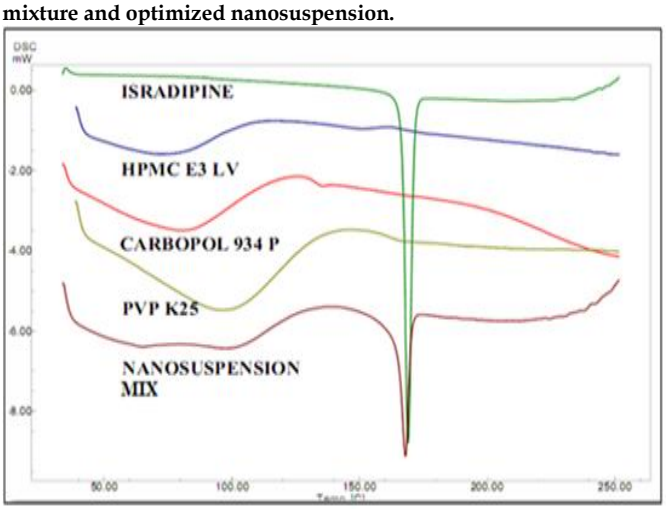

Fig. 11: Overlay of DSC thermograms of Isradipine, HPMC E3 LV, Carbopol 934P, PVP K25 and physical mixture of nanosuspension.

Differential Scanning Calorimetry (DSC)

To characterize the interaction between the drug and stabilizers, DSC study of isradipine pure powder, HPMC E3 LV, Carbopol 934P, PVP K25 and physical mixture of nanosuspension was performed. Differential Scanning Calorimetry studies revealed endothermic or exothermic behavior of substance. Pure isradipine drug showed a sharp peak at 169.04°C which was very much in close to the correspondence of the melting point of drug [16] (Fig. 11).

HPMC E3 LV showed a single melting peak at 72.12°C where as Carbopol 934P showed endothermic peak at 79.9°C and PVP K25 showed endothermic peak at around 96.19°C. [17] Nanosuspension mixture showed sharp endothermic peak at 65.3°C, 97.4°C and 167.9°C which indicated absence of interaction between stabilizers and drug. The melting curves of isradipine nanosuspensions stabilized with various stabilizers were not influenced by stabilizer. So, it can be concluded that there is no interaction between stabilizers and drug.

Stability Study

Mean particle size, drug content and CPR after 90 minutes results of stability samples are recorded in Table 8. For both long term (6 months) and accelerated stability (1 year) studies, the mean particle size of the nanoparticles in the nanosuspension remained small and there was no significant difference found before and after stability of nanosuspension (Table 8).

Similarly, drug content and CPR after 90 minutes of the optimized formulation persisted unchanged confirming the optimum physical stability of the systems. The transmission electron photographs showed that the particles still had a nearly spherical shape and the size of the particles was nearly equal to the size obtained by the laser diffraction method.

Table 8: Stability study for 12 months

Time (month)	Mean particle size (nm) ± S.D*	Drug content ± S.D*	Cpr within 90 minutes ± S.D*
0	248.6 ± 20	99.91 ± 0.08	99.50 ± 0.5
1	254 ± 14	99.54 ± 0.1	99.91 ± 1.2
3	257 ± 17	98.87 ± 1	98.87 ± 1.5
6	263 ± 13	98.56 ± 0.9	98.65 ± 2
12	265 ± 17	98.47 ± 1.3	98.57 ± 1.2

*Results are in mean ± S.D (n=3)

The aim of present work was to formulate nanosuspension containing isradipine, an antihypertensive drug, to improve its dissolution rate and hence, bioavailability. Media milling technique was used as a method of formulation of nanosuspension. The solubility of BCS class II drug, isradipine was enhanced by formulating isradipine nanosuspension by top down technique (Media milling technique). From the literature review, it was found that media milling is best suitable method for formulating nanosuspension compare to precipitation–ultra sonication method because stabilization of nanosuspension is more with media milling technique as compared to precipitation technique and there is no use of harmful organic solvents in it. The stabilizers of nanosuspension were optimized using simplex lattice design where concentration of HPMC E3 LV (X1), Carbopol 934P (X2) and PVP K25 (X3) were selected as independent variables and mean particle size(Y1), saturation solubility(Y2) as well as cumulative percentage drug release after 10 minutes(Y3) were selected as dependent variables. All batches were analysed on basis of mean particle size, drug content, saturation solubility and cpr after 10 min and 90 min. Optimized batch was evaluated for particle size, *in vitro* dissolution, stability study, DSC study, FT-IR and drug content compared with pure drug and concluded there was marked increase in *in vitro* dissolution of nanosuspension compared to pure drug and there was no significant increase in particle size. Thus, nanosuspension is a powerful tool to increase the solubility of poorly water soluble drugs which ultimately leads to enhancement in bioavailability of poorly water soluble drugs.

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