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Research Article

Optimization of Composition and Process for Preparing Metaxalone Nanosuspension using Factorial Design

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ABSTRACT

In the current study, the composition and process for preparing the nanosuspension of metaxalone (MX) has been optimized by using design of experiments (DOE). MX is skeletal muscle relaxant and belongs to BCS class II[1], the class wherein in-vivo drug dissolution is a rate-limiting step for drug absorption[2]. High pressure homogenization (HPH) method was used to prepare the nanosuspension and Hydroxy propyl methyl cellulose (HPMC) and sodium lauryl sulfate (SLS) as surface stabilizers. For optimization studies three square (3^2) factorial design was used. For the composition optimization, concentration of the stabilizers and for process optimization homogenization time and pressure are used as independent variables. The dependent variables were particle size (PS), polydispersity index (PDI), zeta potential (ZP). The relationship between the dependent and independent variables was studied by response surface plots and contour plots. From the data it has been observed that 2.5 % HPMC, 0.5 % SLS were optimum concentrations and 1000 bar pressure, 120 minutes of homogenization were optimum process conditions producing least PS, PDI and high zeta potential. The optimized nano composition prepared by using optimum process conditions was observed to release more than 80 % within 30 minutes and found to be stable after 3 months of storage at room temperature. The solid state characterization (XRD, DSC) data of spray dried nanoparticles of the optimized composition has shown retention of drug crystallinity. IR has shown drug is compatible with the excipients used. SEM photograph has shown spherical drug nanoparticles. The optimization studies by applying the DOE resulted in considerable decrease in the experimentation work to achieve the stable nanosuspension with desired parameters such as PS, PDI and ZP.

Keywords: Metaxalone; nanosuspension; particle size, factorial design.

INTRODUCTION

Aqueous solubility of the drug is one of the important factors to achieve desired concentration of drug in systemic circulation. To achieve the pharmacological activity, the molecules should exhibit certain solubility in physiological intestinal fluids. The aqueous solubility is a major indicator for the solubility of the drug in the intestinal fluids[3]. Poor aqueous solubility is major limiting factor for most of the drugs. Improving the aqueous solubility still remains as a challenging task during development. Common techniques used to enhance the solubility include salt formation, co-solvents, surfactants. micronization, complexation, solid dispersions etc.[4]. Most of these techniques are not universally acceptable because of their own limitations. Nanotechnology is one of the approaches to overcome challenges of conventional drug delivery system based on the development and fabrication of nanostructures. Nanosizing techniques because of their reduction in size have been reported to enhance the dissolution rate by increasing the surface area thereby improving the oral drug bioavailability of poorly water soluble drugs[5,6]. A pharmaceutical nanosuspension is a biphasic system consisting of nanosized drug particles stabilized by surface stabilizers used for oral, topical, parenteral or pulmonary administration[7]. The particle size distribution of the nanoparticles is usually less than 1 micron with an average particle size ranging between 200-600 nm[8]. There are different methods to prepare the nanosuspensions such as precipitation[9], high pressure homogenization[10], wet milling[11]. In this study high pressure homogenization is used to prepare the nanosuspension, because it is most widely used[12,13] due to its advantages such as ease of scale up and little batch to batch variation[14], narrow particle size distribution in final product[15]. Metaxalone is a muscle relaxant used to relax muscles and relieve pain caused by strains, sprains, and other musculoskeletal conditions. Its exact mechanism of action is not known, but it may be due to central nervous system depression. Because of it low solubility and high permeability it belongs to BCS class II drug[16]. Experimental designs such as the factorial designs (FDs) since 1926[17] the screening designs since 1946[18], the central composite designs (CCDs) since 1951[19] and mixture designs (SMDs) since 1958[20] have long been employed to optimize various industrial products. The first literature report on the rational use of optimization appeared in 1967,

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Table 1: Composition Variables (3^2 factorial design).

Batch No.	Drug	Concentration of HPMC		Concentration of SLS	
	%	CV	RV (%)	CV	RV (%)
MX-1A	10	-1	1.25	-1	0.25
MX-1B	10	-1	1.25	0	0.5
MX-1C	10	-1	1.25	+1	0.75
MX-1D	10	0	2.5	-1	0.25
MX-1E	10	0	2.5	0	0.5
MX-1F	10	0	2.5	+1	0.75
MX-1G	10	+1	5	-1	0.25
MX-1H	10	+1	5	0	0.5
MX-1I	10	+1	5	+1	0.275

CV: Coded value; RV: Real value %: Percentage
HPMC: Hydroxy propyl methyl cellulose SLS: Sodium lauryl sulphate

Table 2: Process Variables (3^2 factorial design).

Batch No.	Homogenization Pressure (Bar)		Homogenization time (minutes)	
	CV	RV	CV	RV
MX-2A	-1	750	-1	90
MX-2B	-1	750	0	120
MX-2C	-1	750	+1	150
MX-2D	0	1000	-1	90
MX-2E	0	1000	0	120
MX-2F	0	1000	+1	150
MX-2G	+1	1200	-1	90
MX-2H	+1	1200	0	120
MX-2I	+1	1200	+1	150

when a tablet of sodium salicylate was optimized using an FD[21]. Since then, these systematic approaches have been put into practice in the development of drug formulations at steady pace. Central composite design [CCD] is one of the tools used to investigate the effect of two independent factors. Quality by design refers to the achievement of certain predictable quality with desired and predetermined specifications. Since while preparing the nanosuspensions there are different interacting variables and operating conditions it is important to understand these variables and their interactions. To enable this understanding various statistical experimental designs have been recognized as useful techniques[22]. Optimization through factorial design and response surface methodology is common practice[23,24]. Response surface plots and contour plots describe the influence of the independent variables on the selected responses[25,26]. Factorial design is very useful tool for the identification of critical parameters and to optimize the respective composition and process conditions[27]. The objective of the present study was to apply 3^2 factorial designs to optimize metaxalone nanosuspension and process of preparing the nanosuspension. A 3^2 factorial design was applied to investigate the effect to two composition variables (independent) such as concentration of HPMC and SLS. Similarly, for process optimization two process conditions such as homogenization time and homogenization pressure were used as two independent variables. For both composition and process optimization study, particle size (nm), polydispersity index and zeta potential were taken as responses (dependent variables). Response surface plots and contour plots were drawn and optimization formulation was selected using the desirability function.

MATERIALS AND METHODS

Metaxalone, Hydroxy propyl methyl cellulose (HPMC; supplied by Colorcon), sodium lauryl sulfate (SLS, supplied by JRS), Mannitol from Rouquette Pharma; all other chemicals and solvents were obtained from Dr Reddys laboratories limited. All chemicals and solvents used are of analytical grade. High pressure homogenizer used is FR-756 Model, Panda 2000 Plus.

Compatibility study

Compatibility of the MX with HPMC. SLS, mannitol used to formulate nanosuspension was established by Fourier Transformed Infrared spectral analysis. FT-IR spectral analysis of MX and its physical mixture with HPMC, SLS and mannitol was carried out to investigate any change in chemical composition of the drug after combining it with the excipients

Preparation of drug suspension

The drug suspension was prepared by dissolving weighed quantity of HPMC in 100 ml of purified water. To this, weighed quantity of SLS was added with continuously stirring until clear solution was obtained and avoid foam formation. To this weighed quantity of drug MX was added slowly with continuous stirring. Then finally made up the volume with purified water and stirred for about 15 minutes. Then the drug suspension was subjected to high shear homogenization at 3000 rpm for about 30 minutes to form a uniform dispersion and prevent any lump formation. This drug suspension was further processed by high pressure homogenization process and used for the optimization of the composition as well as the process.

Formulation Optimization

Central composite design was used to optimize and evaluate the main effects of the composition and process parameters on the drug nanoparticles. Further these nanoparticles aggregate and reduce the surface area for wetting and dissolution. Hence stabilizing the nanoparticles by surface stabilizers is required. The amount of the surface stabilizers such as HPMC and SLS was optimized using 2 factor 3 level (3^2) factorial designs. Based on the earlier trial experiments, nine nano compositions were prepared using 3 different concentrations of HPMC and SLS (Table 1). In this study,

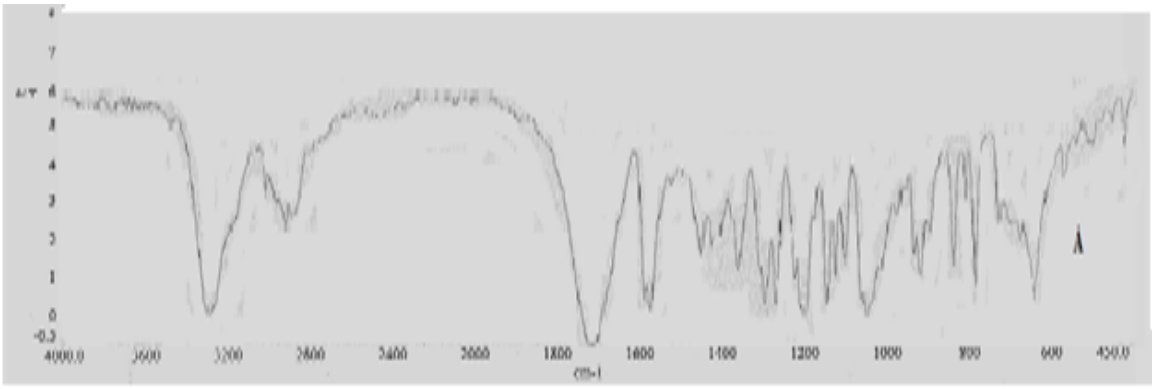


Figure 1: FTIR spectrum of metaxalone.

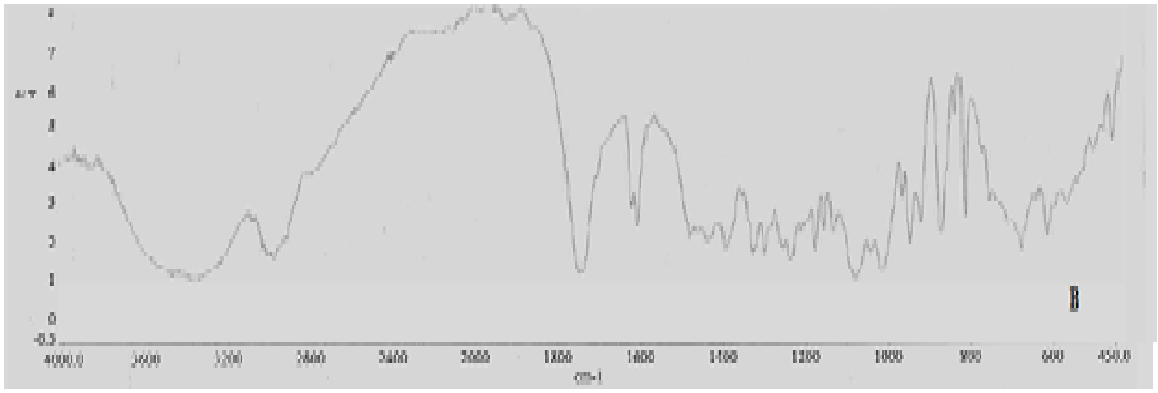


Figure 2: FTIR spectrum of physical mixture of MX, HPMC, SLS, and Mannitol.

Table 3: Experimental results of composition optimization.

	Z-average	Particle size distribution (Intensity)			PDI	ZP
		D10	D50	D90		
MX-1A	399.8	148	205	258	0.6	23.2
MX-1B	324	76.4	179	392	1	29
MX-1C	335	143	195	247	0.7	30.1
MX-1D	114.7	10	156	194	0.25	32
MX-1E	119.5	5.7	84	100	0.1	36
MX-1F	127	7	115	136	0.3	30
MX-1G	251	169	223	267	0.6	23
MX-1H	415.4	82.1	412	717	0.488	22.5
MX-1I	211	12	185	261	0.4	15

the quantity of the drug (10%) and the process conditions were kept constant. Batch size is 150 ml. The prepared drug suspension was subjected to high pressure homogenization at 1000 bar pressure for 90 minutes.

Process Optimization

In the process optimization study, nine compositions were prepared wherein the concentrations were kept constant and process conditions varied in all the nine batches. Drug suspension of 1liter batch size was prepared by using 10% drug, 0.5% SLS and 2.5 % of HPMC. Homogenization pressure and homogenization time were chosen as process variables. Three levels of homogenization pressure namely 800 bar, 1000bar and 1200 bar and three levels of homogenization time is 90 minutes, 120 minutes and 150 minutes (Table 2) were chosen based on trial experiments carried out.

Particle size [PS] and Polydispersity index [PDI]

The particle size and particle size distribution (PSD) affects saturation solubility of nanoparticles. The most widely used method of describing particle size distributions are D values. The D10, D50 and D90 are commonly used to represent the midpoint and range of the particle sizes of a given sample. A D-value can be thought of as a “mass division diameter”. It is the diameter which, when all particles in a sample are arranged in order of ascending mass, divides the sample's mass into specified percentages. The percentage mass below the diameter of interest is the number expressed after the "D". For example the D10 diameter is the diameter at which 10% of a sample's mass is comprised of smaller particles, and the D50 is the diameter at which 50% of a sample's mass is comprised of smaller particles[28]. The Z average is the intensity weighted mean hydrodynamic size of the ensemble collection of particles measured by dynamic light scattering (DLS). The polydispersity index (PDI) is a measure of the distribution of molecular mass in a given polymer sample. PDI gives the physical stability of nanosuspensions and should be as lower as possible for the

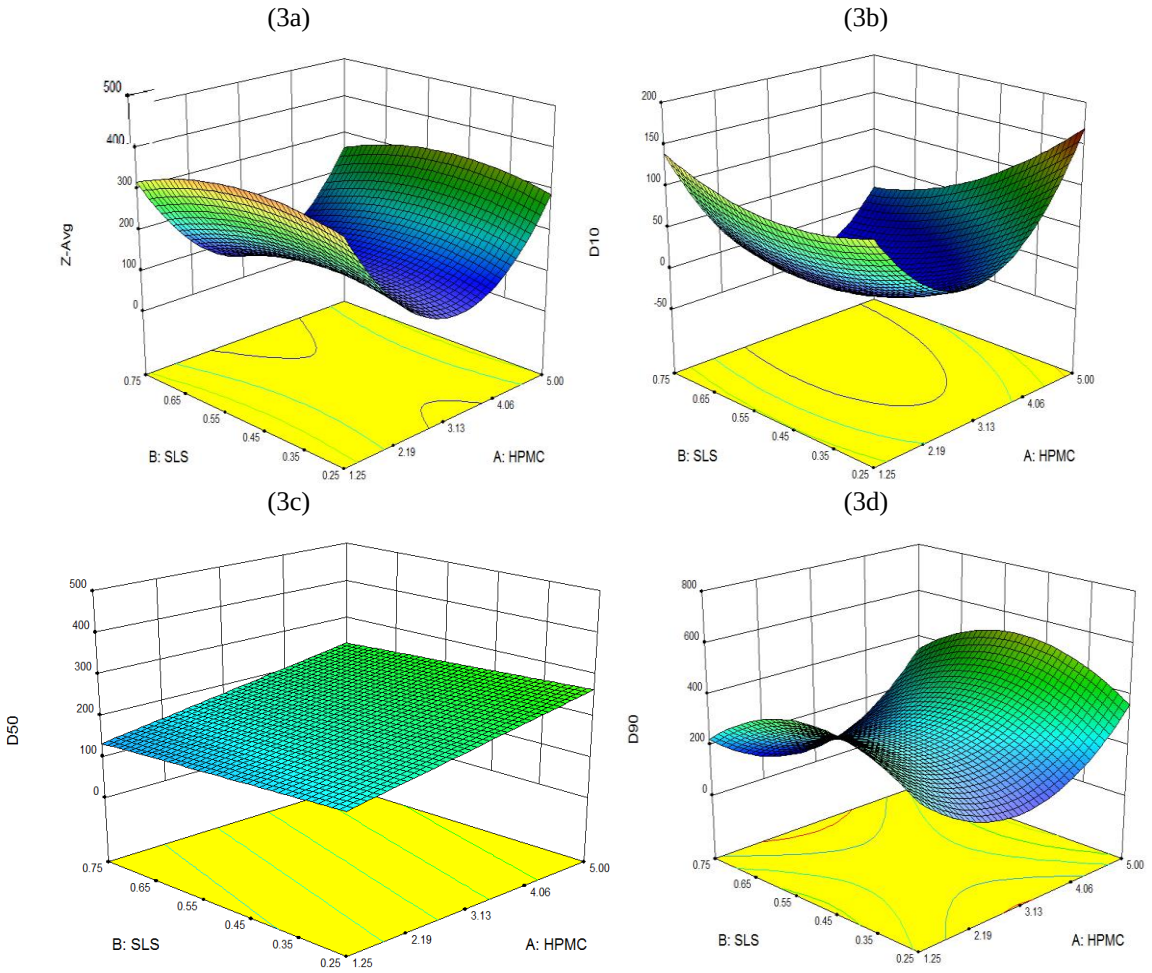


Figure 3: Response surface methodology plot showing effect of HPMC and SLS on particle size 3a) Z-average; 3b) D_{10}3c) D_{50} 3d) D_{90}.

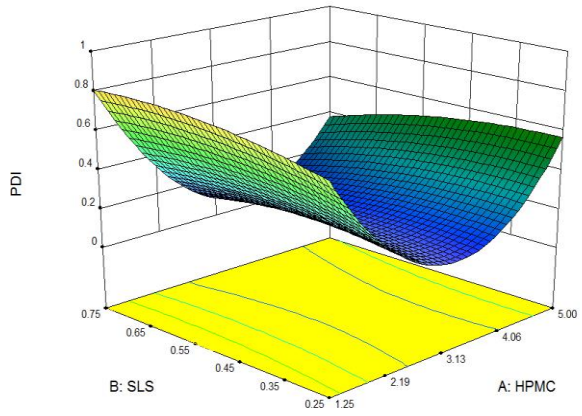


Figure 4: Response surface methodology plot and its contour plot showing effect of HPMC and SLS on PDI.

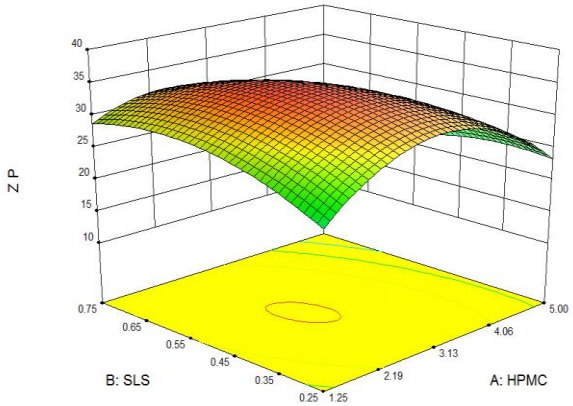


Figure 5: Response surface 3D plot showing effect of HPMC and SLS on ZP.

long-time stability of nanosuspensions. A PDI value of 0.1 to 0.25 shows a fairly narrow size distribution and PDI value more than 0.5 indicates a very broad distribution[29]. The PSD of suspension has been determined for both formulation optimization and process optimization using Malvern Zeta Sizer Nano series nano-ZS. The particle diameter reported was calculated size distribution by intensity. A refractive index of 1.65 has been used for measurements. The PS and PDI has been determined for 9 different batches with different concentration of HPMC or SLS (formulation optimization trials). Similarly, the PS and PDI has been determined for 9 different batches (process optimization trials) homogenized at different pressure (800, 1000, 1200 bar) and different time intervals (90, 120, 150 minutes). The nanosuspension obtained was diluted with water to obtain suitable concentrations for measurement. Diluted nanosuspension was added to the

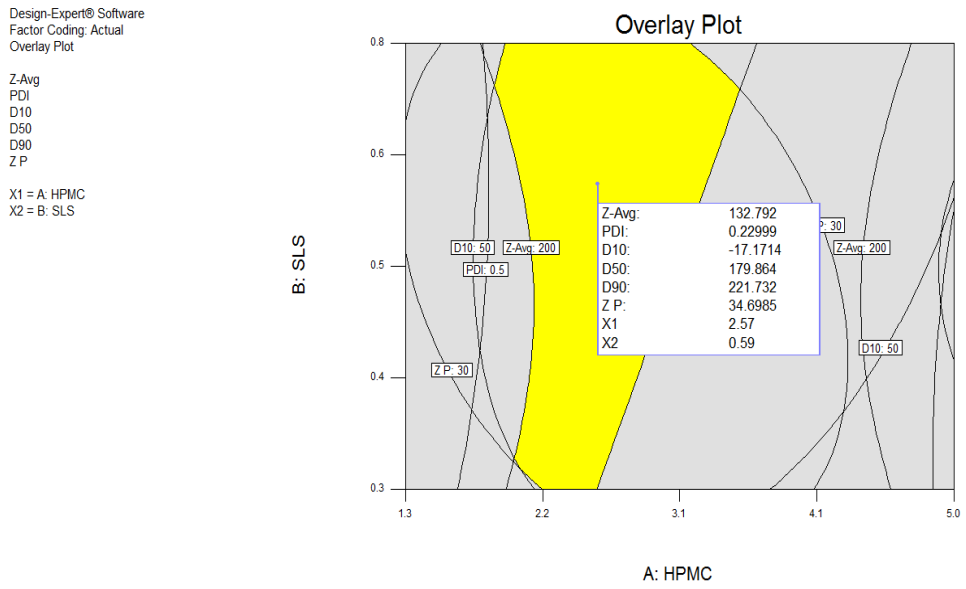


Figure 6: Overlay plot for composition optimization.

Table 4: Experimental results of process optimization.

B.No	Z-average	Particle Size Distribution			PDI	ZP
		D10	D50	D90		
MX-2A	285	89	283	410	0.4	28.5
MX-2B	276.7	37.3	274	518	0.3	32
MX-2C	233	80.7	363	924	0.6	21
MX-2D	231	92.4	102	331	0.2	37
MX-2E	219.9	71	106	322	0.15	37
MX-2F	183.8	67	185	344	0.31	27
MX-2G	275.4	93	231	377	0.5	28.5
MX-2H	481.9	75.5	387	508	0.7	22
MX-2I	584	28.5	349	431	0.8	20

sample cell (quartz cuvette) and put into sample holder unit and measurement was carried out with help of software.

Zeta potential

Zeta potential is a measure of the magnitude of the electrostatic or charge repulsion/attraction between particles, and is one of the fundamental parameters known to affect stability. Its measurement brings detailed insight into the causes of dispersion, aggregation or flocculation, and can be applied to improve the formulation of dispersions, emulsions and suspensions. A prerequisite to achieve an enhancement of oral bioavailability with drug nanoparticles is that nanoparticles are finely dispersed in the gut and do not aggregate. In case they start aggregation, the bioavailability decreases with increasing aggregate formation. This is attributed to the fact that they lose special properties of nanoparticles such as their adhesive property to the mucosal wall. Therefore, it is necessary to prepare nanosuspensions with a physical stability as high as possible. Surface charge properties of the nanosuspensions are studied through zeta potential. The value of particle surface charge indicates the stability of nanosuspensions at the macroscopic level. A minimum zeta potential of ±30 mV is required for electrostatically stabilized nanosuspensions[30,31] and a minimum of ±20 mV for steric stabilization[32]. The zeta potential values are commonly calculated by determining the particle's electrophoretic mobility and then converting the electrophoretic mobility to the zeta potential[33]. Zeta potential of the nano suspension prepared during formulation optimization and process optimization has been analyzed in Malvern zeta sizer after diluting nanosuspension with water to obtain suitable concentration for measurement. Sample was added in specialized zeta cell and the zeta potential measurement was carried out with the help of software.

In-vitro drug release

Drug release of MX from the optimized MX composition was performed in USP dissolution testing apparatus (type II) with rotating paddles at 50rpm using 900ml of 0.5% SLS in water as dissolution medium. The temperature was maintained at 37 ± 0.5 °C throughout the experiment. Samples were estimated by HPLC [Waters Alliance HPLC system, USA) method. Diluent is prepared by mixing buffer: acetonitrile in 65:35 ratio with sonication followed by centrifugation at 4000 rpm for about 10 minutes. Buffer is prepared by dissolving potassium dihydrogen phosphate in water followed by adding triethyl amine and adjusts pH to 2.5±0.05 by phosphoric acid. The solution was filtered through 0.45 µm Durapore PVDF membrane filter and was analyzed by HPLC. The mobile phase is same as diluent.

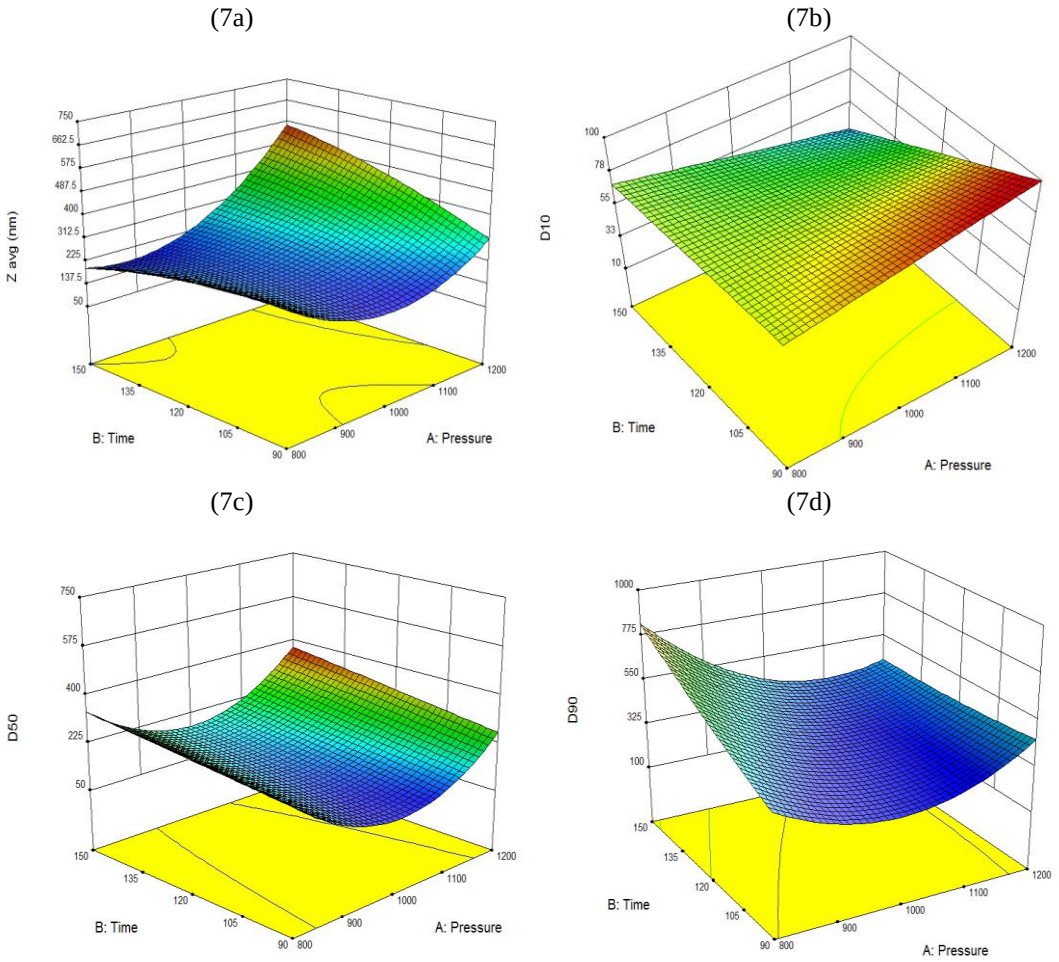


Figure 7: Response surface methodology plots showing effect of independent variables homogenization time and pressure on the dependent variables: Z average (7a); D_{10} (7b); D_{50}(7c); D_{90}(7d).

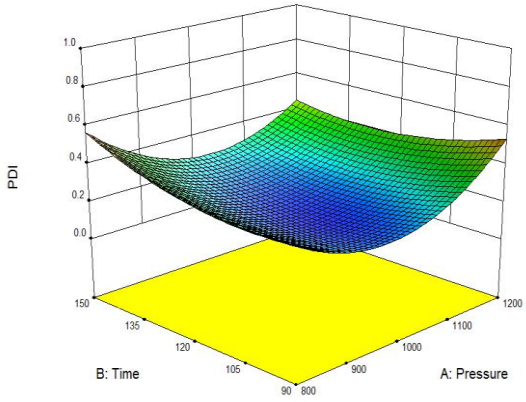


Figure 8: Response surface 3D plots showing effect of independent variables homogenization time and pressure on the dependent variable PDI.

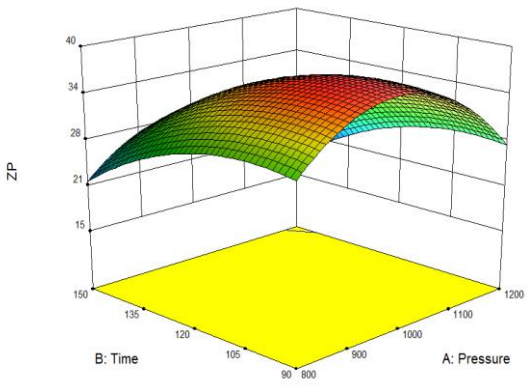


Figure 9: Response surface 3D plots showing effect of independent variables homogenization time and pressure on the dependent variable zeta potential.

Chromatographic separation was accomplished using an150x4.6 mm, X terra RP-8, C-8, 5 µmcolumn. The mobile phase was pumped at a flow rate of 1.0 ml/minute during analysis and maintained at a column temperature of 25 °C and detection wavelength of 230 nm.

Spray drying

The optimized nanosuspension prepared was converted into dry powder using the spray drying in Buchi mini spray dryer using mannitol as redispersant, at inlet temperature of 140 °C, nitrogen pressure 5 kg/cm2and liquid suspension feed rate 6-10 ml/minute. The spray dried nanoparticles is further characterized by XRD, DSC, IR and SEM.

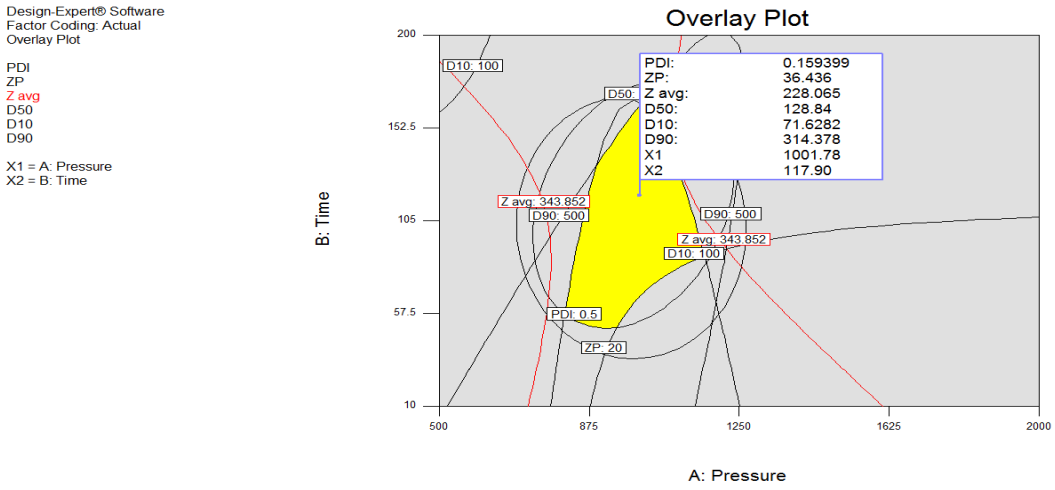


Figure 10: Overlay plot for the process optimization.

Table 5: Optimized composition and process conditions.

	Ingredients	Optimized parameters
Formulation	Metaxalone	10 % w/w
	Hydroxy Propyl methyl cellulose	2.5 % w/w
	Sodium lauryl sulfate	0.5 %w/w
Process	Homogenization pressure	1000 bar
	Homogenization time	120 minutes.

Solid state characterization

Powder X-day diffraction: [PXRD]

Polymorphic or morphological changes of nanosized particles can be checked by assessing the crystalline state and particle morphology[34].As nanosuspension formation experiences high pressure during homogenization, change in crystalline structure of formulation occurs, which may be converted to either amorphous or other polymorphic forms[35,36]. Alteration in the solid state of the drug particles if any and the extent of the amorphous portion is determined by X-ray diffraction analysis[37] and supplemented by differential scanning calorimetry analysis. The X-ray diffractograms of MX, its physical mixture and the spray dried nanoparticles were recorded using a Panalytical Xpert Pro Diffractometer (PANalytical, The Netherlands) with a Cu line as the source of radiation. Standard runs using a 40 kV voltage, a 40mA current and a scanning rate of 0.02°min−1 over a 2θ range of 3 – 45° were used.

Differential scanning calorimetry[38] [DSC]

Thermal characteristics of the MX, physical mixture of MX, HPMC, SLS, mannitol and spray dried nanoparticles was studied. Thermal properties of powder samples were investigated using a Perkin-Elmer DSC-7 differential scanning calorimeter / TAC-7 thermal analysis controller with an intracooler-2 cooling system (Perkin- Elmer Instruments, USA). For evaluation about 3 to 5 mg of MX or physical mixture/spray dried nanoparticles was placed in perforated aluminum sealed 50 µL pans and the heat runs for each sample was set from 20 to 200°C at 10°C/minute, an inert environment was maintained using nitrogen.

Fourier transform Infra –Red Spectroscopy [FTIR]

The infrared spectra are recorded on Fourier Transform Spectrometer in the mid–infrared region (MIR) within the range (400-4000 cm-1). Due to the complex interaction of atoms within the molecule, IR absorption of the functional groups may vary over a wide range. However, it has been found that many functional groups give characteristic IR absorption at specific narrow frequency range. Multiple functional groups may absorb at one particular frequency range but a functional group often gives rise to several characteristic absorptions. Stretching an bending vibrations are varied after formulation can be observed. Thus, the spectral interpretations should not be confined to one or two bands only actually the whole spectrum should be examined[39,40]. FT- IR spectra of MX [Figure 1], physical mixture of MX with excipients used HPMC, SLS, mannitol [Figure 2] and spray dried nanoparticles [Figure 14] were recorded on the sample prepared in KBr disks, wherein sample and KBr are taken in 1:100 ratio) using Shimadzu Fourier Transform Infra-Red spectrometer. The samples were scanned over a frequency range 4000-400 cm-1.

Scanning electron Microscopy

Scanning electron microscopy is a type of electron microscopy that images the surface of solid specimen by using focused beam of high-energy electrons. Scanning electron microscopy (SEM) is giving morphological examination with direct visualization. The techniques based on electron microscopy offer several advantages in morphological and sizing analysis. For SEM characterization dry powder is mounted on a sample holder followed by coating with a conductive metal. The sample is then scanned with a focused fine beam of electrons[41]. The electrons which are scattered and/or generated through secondary processes, are collected through secondary electron or back-scattered electron detectors. The backscattered electron images are sensitive to the atomic weight of the elements present. The regions of the image which appear brighter indicate the presence of high atomic weight elements. The surface characteristics of the sample

Table 6: Data generated for Optimized batch.

	Z-average	Particle Size Distribution			PDI	ZP
		D10	D50	D90		
Initial	121	53	108	274	0.18	37
1 month	144	61	101	293	0.2	36
2 month	137	43	116	281	0.2	37
3 month	130	38	103	296	0.2	35

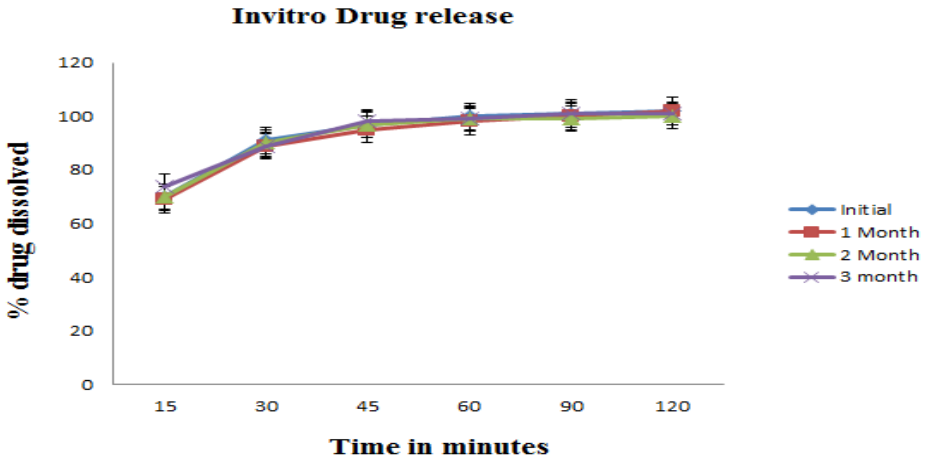


Figure 11: In-vitro release profile of Optimized batch at initial and stability conditions.

are obtained from the secondary electrons emitted from the sample surface. The nanoparticles must be able to withstand vacuum sometimes the electron beam may damage the polymer[42]. MX, physical mixture or spray dried nanoparticles was placed on the Carbon tape stuck to the Aluminum SEM stub. Later it was imaged in the SEM at a low vacuum.

RESULTS AND DISCUSSION

Drug-excipients compatibility studies

To study the compatibility of drug with excipients, IR spectra analysis of pure drug MX and physical mixture of drug MX with all the excipients such as SLS, HPMC, mannitol was studied. IR spectra of MX were shown in Figure 1; Figure 2 shows IR spectra of physical mixture and Figure 14 shows IR spectra of the spray dried nanoparticles. From figure 1 and 2 it has been shown that there is slight broadening of the peaks and no significant physical and/or chemical interaction in between drug and studied excipients. The frequencies of functional groups of drug quetiapine remained intact in physical mixture. So it was concluded that there was no major interaction occurred and are compatible.

Composition optimization.

The aim of the 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. All the measured responses that may affect the quality of the product were taken into consideration during the optimization procedure. Evaluation of MX-1A to MX-1I was done by determining Z-average, Particle size distribution (PSD), poly dispersity index and zeta potential for all the compositions as shown in table 3. Further various response surface methodology (RSM) plots and 2D contour plots for the composition optimization study were performed employing Design-Expert software (Version 9.0.1.0, Stat-Ease Inc., Minneapolis, MN). The significance of these parameters on the variables was assessed by analysis of variance (ANOVA, 2-way).

Particle size

Mean particle size (MPS) of the formulation for different batches was found between 84 nm to 412 nm. Figure 3 represents the response surface methodology (RSM) plot and contour plots for particle size distribution data including Z-average, D_{10}, D_{50}, D_{90}. From Figure 3a, it has been observed that with increase in the concentration of SLS, Z-average almost remained constant and with increase in concentration of HPMC the particle size decreased upto midway concentration and then gradually increased with increase in the concentration. At low or high concentration of HPMC the effect of SLS concentration on particle size remained constant. Similarly, at low or high concentration of SLS the effect of HPMC on particle size is same, which initially decreases and then increases with high variability at high concentration of HPMC when compared to low concentration. Similar to Z-average with increase in concentration of HPMC; particle size ie D10 [Figure 3b] decreased up to mid-point concentration and then gradually increases with further increase in the concentration of HPMC with high variability at high concentration of HPMC. At high concentration of HPMC with the increase in concentration of SLS the particle size is reduced with high variability at low concentration of SLS. Similarly, at low concentration of HPMC the increase in concentration of SLS, particle size remained constant up to midway concentration and then increase with high variability at high concentration of SLS. With respect to

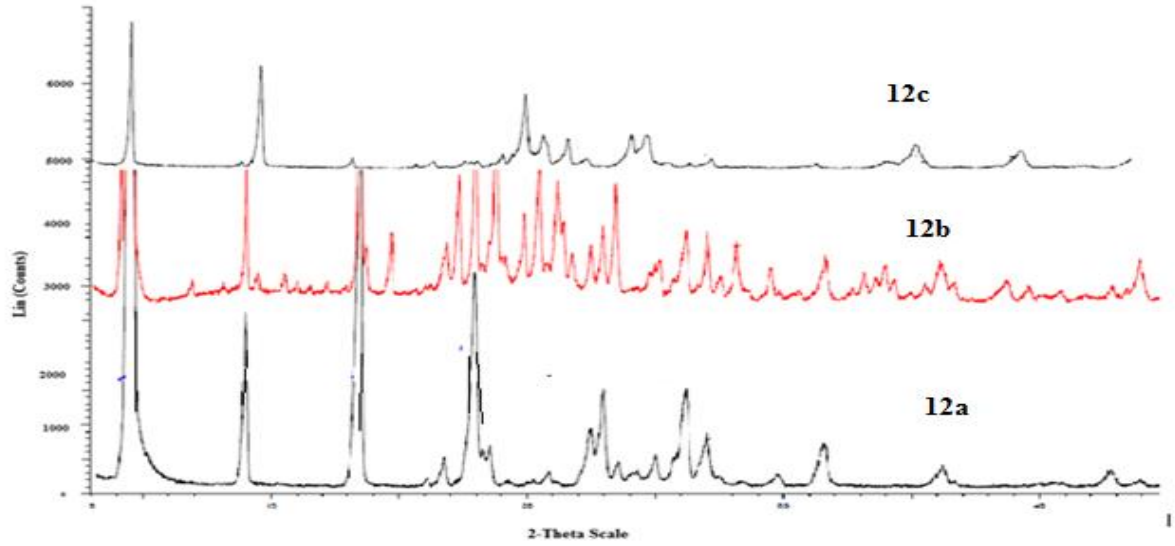


Figure 12: shows the XRD of the MX (12a), physical mixture (12b)and spray dried powder (12c) of optimized nanosuspension.

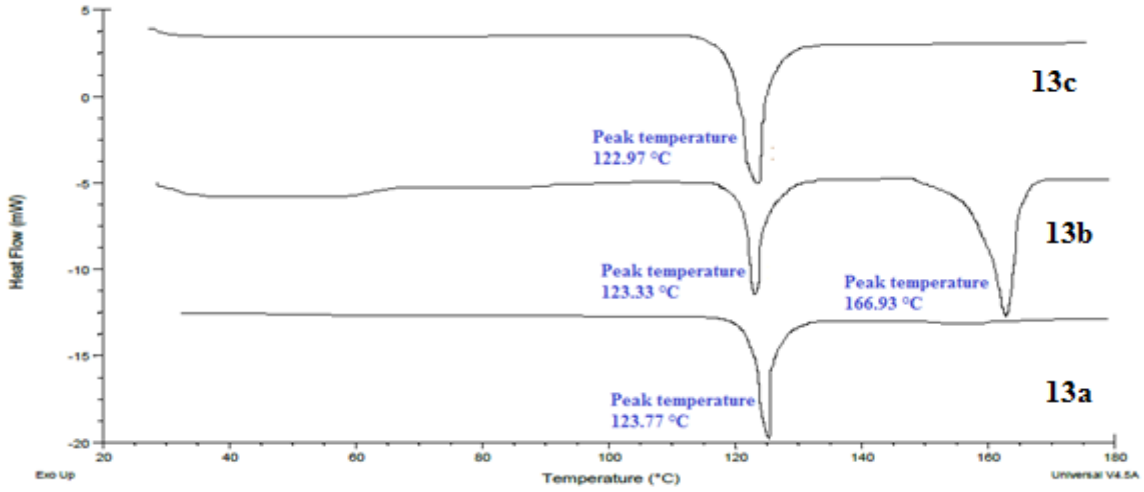


Figure 13: shows DSC plot of MX (13a), physical mixture of MX,HPMC,SLS,mannitol (13b) and spray dried nanoparticles(13c).

D50 [figure 3c], it has been observed that with increase in concentration of HPMC /SLS the particle size is increased. The increase in particle size is less for SLS than that observed with concentration of HPMC. At high concentration of HPMC it is almost constant irrespective of concentration of SLS. And for D90 [Figure 3d], the RSM plot shows that effect of SLS at low and high concentration of HPMC remains same, wherein with increase in concentration of SLS, the particle size slightly increases up to midway and then decreases. Irrespective of SLS concentration, increase in concentration of HPMC the particle size gradually decreases and then drastically increase with high variability at high concentration of HPMC.

Polydispersity index

The combined effect of SLS and HPMC on the PDI was studied using the response surface methodology. Figure 4 shows the RSM plot of the PDI and its contour plot as measure for the particle size distribution in response to the investigated factors. PDI shows more dependence on concentration of HPMC. With the increase in the concentration of HPMC, PDI decreases upto midway concentration and then increases. At lower concentration of HPMC, with increase in concentration of SLS, the PDI also increases, however at high concentration of HPMC the PDI slightly decreases with increase in concentration of SLS. At mid-point concentration of HPMC, PDI almost remained constant with increase in the concentration of SLS.

Zeta potential

From the Figure 5 it has been observed that with increase in the concentration of HPMC, ZP increased up to certain point and then gradually decreased. And with increase in the concentration of SLS, ZP also increased at low

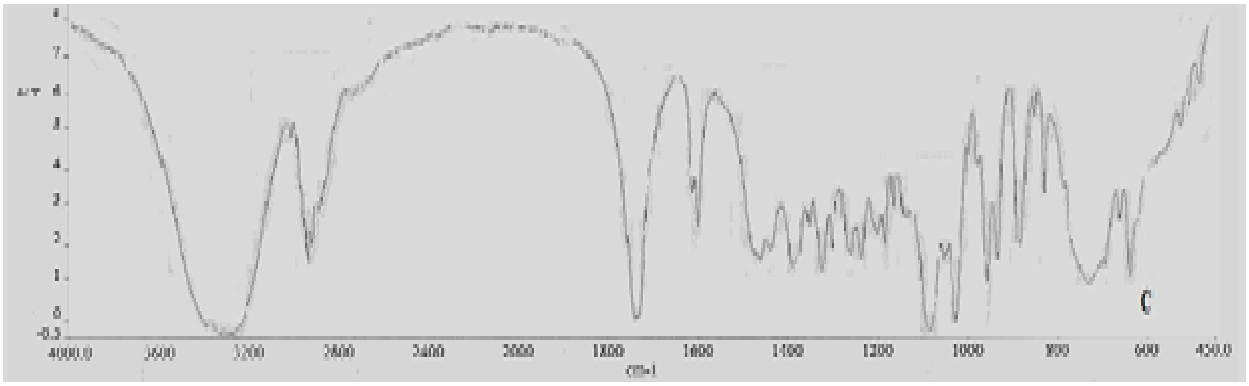
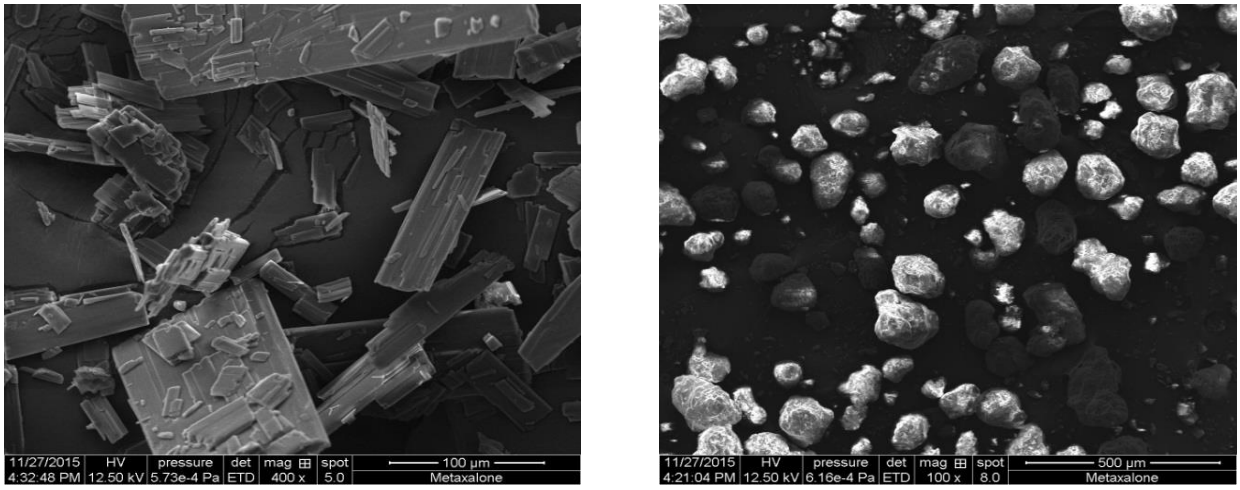


Figure 14: FTIR spray dried nanoparticles.



(15a) (15b)

Figure 15: SEM photograph of the MX (15a); spray dried powder of the optimized composition (15 b).

concentration of HPMC and from mid-point concentration to high concentration of HPMC, ZP almost remained constant with increase in the concentration of the SLS. From the formulation optimization study MX-1E batch was found to be optimized composition having 2.5% of HPMC concentration and 0.5 % SLS concentration. Overlay plot was prepared using Design Expert 9.0.1 software. From the overlay plot particle size Z-average of 132 nm PDI of 0.2 zeta potential of 34 should come at the 2.57% HPMC concentration and 0.59 % SLS concentration. Figure 6 represents the overlay plot for the optimization of the composition.

Process optimization study

Nine compositions MX-2A to MX-2I were prepared and studied for process optimization. In all the batches the concentration of drug (10%), HPMC (2.5 %) and SLS concentration (0.5 %) was kept constant. The data generated for PS, PDI and ZP was captured in table 4. To further analyze the effect of the variables on the responses, RSM plots and its contour plots were generated. The relationship between the dependable variables and the two independent variables was further elucidated by constructing these plots.

Particle size

The effect of the homogenization time and pressure on the particle size has been studied and the RSM plots along with their contour plots have been captured in figure 7a to 7d. From response surface plot of Z-average [Figure 7a] it has been observed that at low pressure the particle size almost remained constant with increase in homogenization time, however at high pressure Z-average increase with increase in time. Irrespective of time, Z-average initially decreased and then increase with high variability at high homogenization time and high homogenization pressure. With respect to D10 [figure 7b] at low pressure and time minimum particle size observed with high variability. With increase in pressure and time, the particle size also increased. However, at low and high pressure/time there was high variability observed. From Figure 7c, it has been observed that irrespective of time, with increase in the pressure particle size (D50) initially reduced and then increased. At low pressure and high time, the variability in particle size in observed. At high pressure, particle size increase with increase in the time. For d90 [figure 7d] with increase in the pressure the particle size initially decreased and then increases. At low pressure with increase in time, particle size increases with high variability at high time. At high pressure particle size almost remained constant with respect to time. At high time, the particle size reduces drastically up to mid –point of pressure and then slightly increases.

Polydispersity index [PDI]

From the Figure 8 it has been observed that the PDI is affected by homogenization pressure than homogenization

time. PDI value remained almost constant with respect to homogenization time. However with increase in the pressure, PDI value decreases and then gradually increased, irrespective of time. The batch with 1000 bar after 120 minutes of homogenization showed the least PDI value 0.15, owing to the narrower particle size distribution.

Zeta potential

From the Figure 9 it has been observed that ZP is affected by both pressure and time. With increase in the homogenization pressure ZP also increases and then gradually decreases. At with increase in the time, ZP increased up to certain point and then gradually decreased with increase in time. The highest zeta potential has been observed for 1000 bar pressure and 90-120 minutes of homogenization time, however at low time, variability is observed. From the process optimization study MX-2E batch was found to be optimized composition with 1000 bar and 120 minutes as homogenization pressure and time respectively. Overlay plot was prepared using Design Expert 9.0.1.0 software as shown in Figure 10. From the overlay plot particle size d50 of 128 nm; PDI 0.15; zeta potential 36 should come at 1000 homogenization pressure and 117 minutes of homogenization. Figure 10 represents the overlay plot for the process optimization.

Reproducibility and stability

Nanosuspension with the optimized concentration of the HPMC and SLS was prepared with optimized homogenization pressure and time as shown in table 5 at 1liter batch size. This optimized composition was stored at room temperature (RT) for about 3 months and analyzed for particle size, PDI, zeta potential, in-vitro release profile at initial and after storage for about 3 months. Table 6 shows the data generated for the optimized batch at initial and on stability at RT which was matching with that of data obtained during optimization studies. Further it has been observed that reproducible results were obtained after 1M, 2M and 3M storage at RT when compared to that of initial results. Figure 11 shows the in-vitro release profile of optimized composition at initial, 1month, 2 months, 3 months storage at RT.

Solid State Characterization

The optimized nanosuspension was converted into solid powder by spray drying. The spray dried nanoparticles were further characterized for XRD, DSC, IR and SEM analysis. Figure 12a, 12b, 12c and figure 13a, 13b, 13c represent the XRD and DSC of MX, physical mixture of MX, HPMC, SLS, mannitol and spray dried nanoparticles respectively. Figure 14 represent the IR spectra of spray dried nanoparticles. Figure 15a and 15b represent the SEM photographs of MX and spray dried nanoparticles. From this data it has been observed that drug after reduction of the particle size to nano size also the drug retained the crystalline peaks thus retained its crystalline nature. SEM photograph showed how the irregular shaped elongated particles of metaxalone have been converted into spherical particles during spray drying. The spray dried particles show the deposition of the surface stabilizers along with redispersants onto the drug nanoparticles thus creating the hydrophilic microenvironment.

CONCLUSION

A DOE was performed to optimize the composition, process and study the effect of the composition and process conditions on the response variables. Drug excipient compatibility was established by the infra-red analysis. It has been observed that the both concentration of the surface stabilizers and the process conditions had shown effect on the critical parameters of the nanosuspension such as particle size, poly dispersity index and the zeta potential. From the analysis of the particle size data of formulation optimization study, it has been observed that at the particle size is mainly affected by concentration of HPMC. At lower and higher concentration of HPMC the particle size is high with high variability. Like particle size, PDI is also dependent on HPMC concentration and Zeta potential is affected by both HPMC and SLS concentration. Similarly from the process optimization study variability has been observed at high pressure and time. And at low to medium pressure the particle size remained constant even with increase in time, however at high pressure it increases with increase in time. Particle size values such as Z-average and D90 has shown high variability at high pressure and high homogenization time respectively. PDI is mainly affected by pressure. Zeta potential is affected by both pressure and time where with increase in pressure and time, zeta potential increased and then decreased subsequently. With the DOE optimization study of the composition and process, the concentrations 2.5% HPMC and 0.5% SLS, process conditions such as 1000 bar homogenization pressure and 120 minutes of homogenization time have found to achieve desired particle size, polydispersity index, zeta potential values. From the solid characterization data for dried nanoparticles, retention of drug crystallinity has been observed after subjecting to spray drying process which was shown by the XRD and DSC analysis. SEM photographs have shown how the irregular shaped metaxalone drug particles have been converted into spherical particles with surface stabilizers deposited around drug to give spherical shape and hydrophilic microenvironment. The drug nanoparticles have shown more than 80 % of drug release in about 30 minutes. Metaxalone nanoparticles were found to be stable after 3 months of storage period at room temperature.

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