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Formulation and Process Optimization of Quetiapine Fumarate Nanosuspension using Factorial Design

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ABSTRACT

In the present study, the optimization of composition and process for preparation of the nanosuspension of quetiapine fumarate (QF) was carried out by using design of experiments (DOE). Quetiapine fumarate (QF) is atypical antipsychotic drug under BCS class II. Due to its poor aqueous solubility, the oral bioavailability is only 9 %. High pressure homogenization (HPH) was used as technique for preparing the nanosuspension. For optimization of the composition and process of QF nanosuspension, the three square (3^2) factorial design was used. For the composition optimization, concentration of the Polyvinyl pyrrolidone (PVP), sodium lauryl sulphate (SLS) and for process optimization homogenization time, homogenization pressure were 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 further elucidated by response surface plots and contour plots. From the analysis of the data it has been observed that 5.25 % PVP, 0.75 % SLS were optimum concentrations and 750 bar pressure, 90 minutes of homogenization were optimum process conditions. The optimized nano composition prepared using optimized process conditions for preparing QF nanosuspension 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 loss of drug crystallinity. IR has shown drug is compatible with the excipients used. SEM photograph of the spray dried nanoparticles of optimized composition has shown spherical drug nanoparticles. The optimization of the composition and homogenizing process 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: Quetiapine Fumarate; nanosuspension; particle size, factorial design.

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INTRODUCTION

A pharmaceutical nanosuspension is a biphasic system consisting of nanosized drug particles stabilized by polymeric surfactants used for oral, topical, parenteral or pulmonary administration[1]. The particle size distribution of the solid nanoparticles in nanosuspension is usually less than 1 micron with an average particle size ranging between 200-600 nm[2]. To achieve the pharmacological activity, the molecules should exhibit certain solubility in physiological intestinal fluids. An improvement in the oral bioavailability of poorly water soluble drugs remains as one of the most challenging aspect of drug development. The aqueous solubility is a major indicator for the solubility of the drug in the intestinal fluids[3] There are many drugs of various therapeutic categories that fall in BCS class II or IV as they lack solubility. There are various techniques available to improve the solubility of poorly soluble drugs. Some of the approaches include micronization, nanonization, solid dispersions, complexation, use of permeation enhancers and salt formation[4] etc. Most of these techniques have their own limitations; hence have limited utility in solubility enhancement. Nanotechnology is one approach to overcome challenges of conventional drug delivery systems based on the development and fabrication of nanostructures[5] such as nanoparticles. Nanosuspensions can successfully formulate the poorly soluble drugs for improved dissolution and absorption. The important feature of the nanosuspension is their ability to improve saturation solubility and consequently dissolution rate of the specific drug. Further the nanosuspensions have advantage of high drug loading. Quetiapine Fumarate is atypical antipsychotic drug used to treat schizophrenia and bipolar disorder. The oral bioavailability of quetiapine fumarate is only 9%. In addition to the traditional experimentation, factorial design is very useful tool for the identification of critical parameters and to optimize the respective composition and process conditions[6]. The interaction between the two factors such as concentration of polymer and surfactant can be systematically examined by applying two full factorial design. The particle sizes, Polydispersity index, zeta potential were investigated as responses describing the quality of the resulting nanosuspensions. Experimental designs have long been employed to optimize various industrial products and/or processes such as the factorial designs (FDs) since 1926,[7] the screening designs since 1946[8], the central composite designs (CCDs) since 1951[9] and mixture designs (SMDs) since 1958[10]. The use of optimization techniques employing design of experiments (DoE), however, permeated the field of pharmaceutical product/process development around four decades ago. The first literature report on the rational use of optimization appeared in 1967, when a tablet of sodium salicylate was optimized using an

FD[11] 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 study the effect of different variables on the dependent variables of any formulation. Based on the principal design of the experiments, CCD was employed to investigate the effect of two independent factors. The aim of the present study is to prepare and evaluate eighteen compositions of quetiapine nanosuspension (nine for composition optimization and nine for process optimization). A two factor three level factorial design was used for obtaining a prediction of the optimized formulation. Further the purpose of this study was to optimize the high pressure homogenization process for preparing the nanosuspension. The aim of the pharmaceutical formulation and process optimization is generally to find the levels of the variable that affect the chosen responses and determine the levels of variable from which a robust product with high quality characteristics may be produced[12].

MATERIALS AND METHOD

Quetiapine fumarate, polyvinyl pyrrolidone (PVP; supplied by BASF), sodium lauryl sulfate (SLS, supplied by JRS) and 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 Quetiapine fumarate with polyvinyl pyrrolidone and sodium lauryl sulfate used to formulate nanosuspension was established by Fourier Transformed Infrared spectral analysis. FT-IR spectral analysis of Quetiapine fumarate and its physical mixture with polyvinyl pyrrolidone and sodium lauryl sulfate 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 polyvinyl pyrrolidone in purified water. To this, weighed quantity of sodium lauryl sulfate was added with continuously stirring until clear solution was obtained. To this weighed quantity of drug quetiapine fumarate was added with continuous stirring. Then finally made up the volume to with purified water and stirred for about 10 minutes. Then the drug suspension was subjected to high shear homogenization at 4000 rpm for about 10-15 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 PVP K-30 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 PVP and sodium lauryl sulfate (Table 1). In this study, the quantity of the drug (5%) 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 60 minutes.

Table 1: Formulation Variables (3^2 factorial design)

	Drug		Concentration of PVP			Concentration of SLS		
	%	Qty	CV	RV (%)	Qty (g)	CV	RV (%)	Qty (g)
QF-9A	5	7.5	-1	3	4.5	_1	0.5	0.75
QF-9B	5	7.5	-1	3	4.5	0	0.75	1.125
QF-9C	5	7.5	-1	3	4.5	+1	1	1.5
QF-9D	5	7.5	0	5.25	7.875	_1	0.5	0.75
QF-9E	5	7.5	0	5.25	7.875	0	0.75	1.125
QF-9F	5	7.5	0	5.25	7.875	+1	1	1.5
QF-9G	5	7.5	+1	7.5	11.25	_1	0.5	0.75
QF-9H	5	7.5	+1	7.5	11.25	0	0.75	1.125
QF-9I	5	7.5	+1	7.5	11.25	+1	1	1.5

Qty: Quantity; CV: Coded value; RV: Real value: gram%: Percentage PVP: Polyvinyl pyrrolidone SLS: Sodium lauryl sulphate

Process Optimization

In the process optimization study, the composition was kept constant and process conditions varied in all the nine batches. The drug suspension was prepared by using 5% drug, 1% sodium lauryl sulfate and 2.5 % of poly vinyl pyrrolidone. Batch size was 1 liter. Homogenization pressure and homogenization time were chosen as process variables. Three levels of homogenization pressure namely 500 bar, 750 bar and 1000 bar and three levels of homogenization time is 30 minutes, 60 minutes and 90 minutes (Table 2) were chosen based on earlier trial experiments carried out.

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

	Homogenization Pressure (Bar)		Homogenization time (minutes)	
	CV	RV	CV	RV
QF-8A	-1	500	-1	30
QF-8B	-1	500	0	60
QF-8C	-1	500	+1	90

QF-8D	0	750	-1	30
QF-8E	0	750	0	60
QF-8F	0	750	+1	90
QF-8G	+1	1000	-1	30
QF-8H	+1	1000	0	60
QF-8I	+1	1000	+1	90

Particle size [PS] and Polydispersity index [PDI]

The particle size and particle size distribution (PSD) affects saturation solubility of nanoparticles. The particle size distribution and its range named PDI can be determined by laser diffraction (LD), photon correlation spectroscopy, microscope, and coulter counter [13]. PDI gives the physical stability of nanosuspensions and should be as lower as possible for the 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[14]. 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 PVP or SLS (formulation optimization trials) taken after homogenizing for 60 minutes at 1000 bar pressure. Similarly the PS and PDI has been determined for 9 different batches (process optimization trials) homogenized at different pressure (500, 750, 1000 bar) and different time intervals (30, 60, 90 minutes). The nanosuspension obtained was diluted with water to obtain suitable concentrations for measurement. Diluted nanosuspension was added to the sample cell (quartz cuvette) and put into sample holder unit and measurement was carried out with help of software.

Zeta Potential

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 nanosuspensionsn[15,16] and a minimum of ±20 mV for steric stabilization[17] The zeta potential values are commonly calculated by determining the particle's electrophoretic mobility and then converting the

electrophoretic mobility to the zeta potential[18] 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 quetiapine from the optimized QF nano suspension was performed in USP dissolution testing apparatus (type II) with rotating paddles at 50rpm using 900ml of pH 6.8 phosphate buffer 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. The mobile phase consists of mixture of potassium dihydrogen phosphate buffer, acetonitrile, triethyl amine pH adjusted to 6.7 with KOH solution. Chromatographic separation was accomplished using an Xterra Column RP8 5 µm; 4.6X150 mm column. The mobile phase was pumped isocratically at a flow rate of 1.0 mL/minute during analysis and maintained at a column temperature of 40°C.

Spray drying

The optimized nanosuspension prepared was converted into dry powder using the spray drying in Buchi mini spray dryer, with an inlet temperature of 140 °C and feed rate of 5 ml per minute. The spray dried nanoparticles is further characterized by XRD, DSC, IR and SEM.

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[19]. A nanosuspension formation experiences high pressure during homogenization, change in crystalline nature of drug or active pharmaceutical ingredient (API)" may occur, which may convert drug or API to either amorphous or other polymorphic forms[20,21] Alteration in the solid state of the drug particles and the extent of the amorphous portion is determined by X-ray diffraction analysis[22] and supplemented by differential scanning calorimetry analysis. The X-ray diffractograms of QF 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[23] [DSC]

Thermal characteristics of the QF, 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 QF or 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 & 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[24,25] FT-IR spectra of QF [Figure 1], physical mixture of QF with excipients PVP, SLS [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, spray dried nanoparticles should be first converted into a dry powder, which is then mounted on a sample holder followed by coating with a conductive metal. The sample is then scanned with a focused fine beam of electrons[26].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 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[27]. Quetiapine Fumarate 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 QF and physical mixture of drug QF with all the excipients such as SLS, PVP in 1:1 ratio was studied. IR spectra of QF was shown in Figure 1 and Figure 2 shows IR spectra of physical mixture. From figure 1 and 2 it has been shown that there is 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.

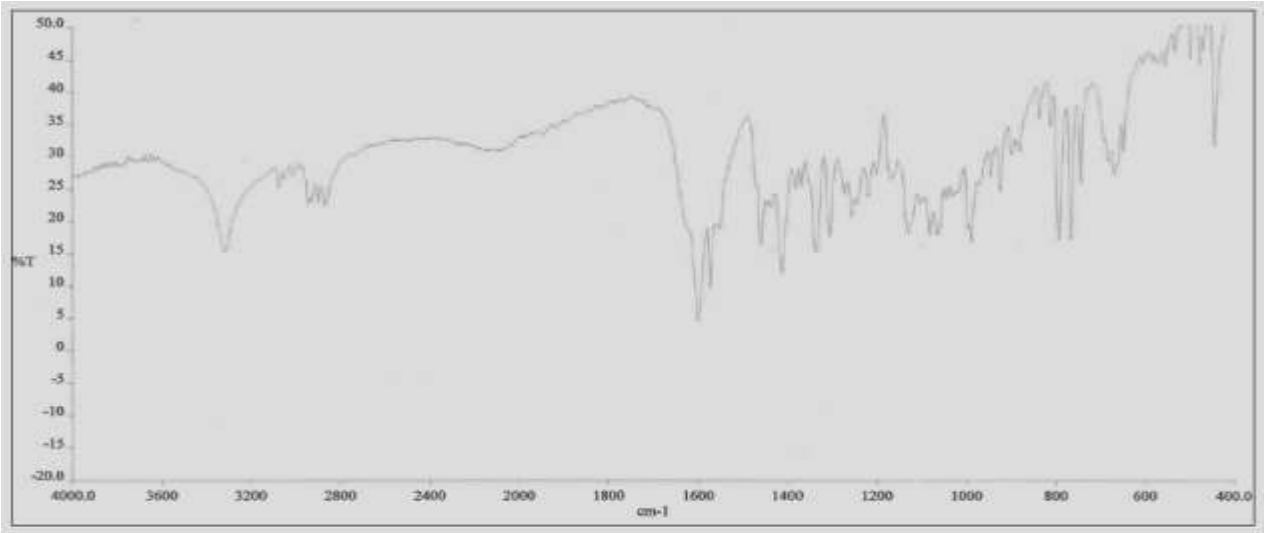


Figure 1: FTIR spectrum of Quetiapine Fumarate

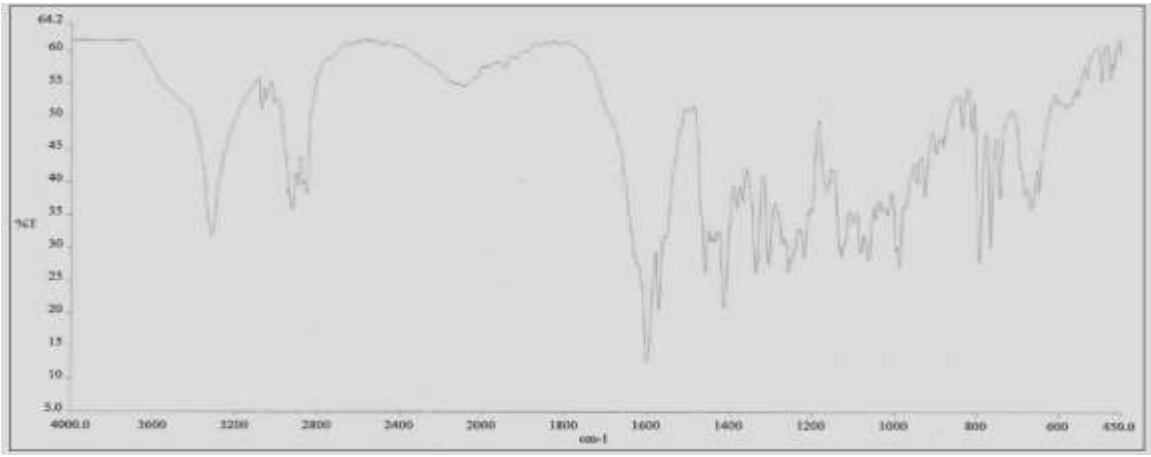


Figure 2: FTIR spectrum of physical mixture of quetiapine fumarate, Polyvinyl pyrrolidone and Sodium lauryl sulfate

Formulation 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 QF-09A to QF-09I was done by determining Z-average, Particle size distribution (PSD), poly disperisty index, zeta potential for all the compositions as shown in table 3.

Table 3: Experimental results of formulation optimization

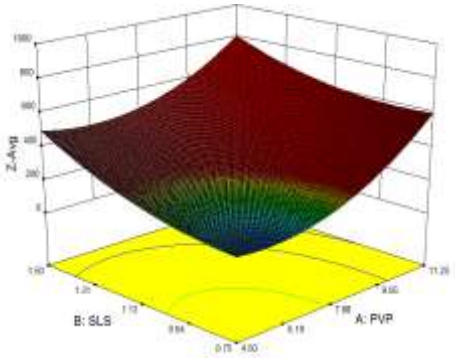
B. No	Z-average	Particle size distribution (Intensity)			PDI	ZP
		D10	D50	D90		
QF-09 A	230	19.1	310	410	0.4	28.9
QF-09 B	215	24	137	134	0.5	32.2
QF-09 C	275	29	154	190	0.9	36
QF-09 D	167	19	144	184	0.5	27.5
QF-09 E	127	17.8	136	181	0.4	32.8
QF-09 F	194	15.6	190	286	0.6	28.5
QF-09 G	234	11.5	185	261	0.3	22.7
QF-09 H	215	13.1	158	229	0.5	27.5
QF-09 I	216	22	340	565	0.5	26.9

Further various response surface methodology (RSM) computations 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).

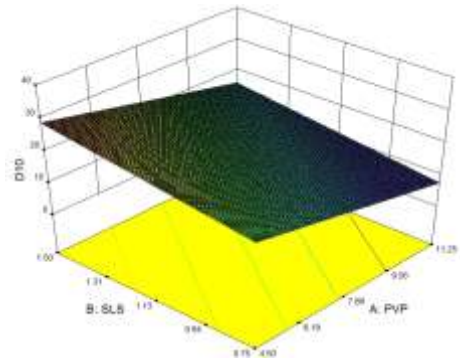
Particle size

Particle size of the formulation for different batches was found between 100 nm to 600 nm. In figure 3a to 3d the response surface depicts the effect of the amount of the stabilizers SLS and PVP at different levels on the particle size. From the response surface and contour plots of d50, d90 and Z-average it has been observed that with increase in the concentration of both steric stabilizer i.e. PVP and electrostatic stabilizer i.e SLS particle size was also increased, however the particle size at the low to medium concentrations of both the stabilizers have been observed to be almost constant and with further increase in the quantity of the stabilizers from medium to high concentration the particle size was also found to be increased. At lower concentration of stabilizers though the particle size observed to be low but a high variability has been observed which is evident from the response surface plots of D50, D90 and Z-average. With respect to D10 value at lower concentration of stabilizers the particle size and the variability also low. The formulation

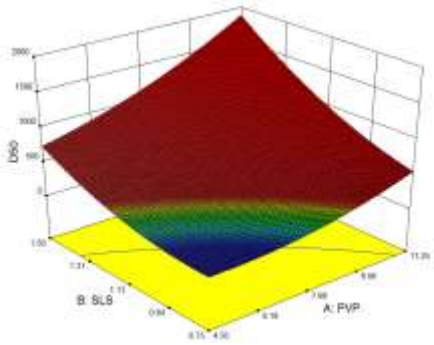
with 5.25 % of PVP concentration and 0.75 % of SLS concentration showed the lowest particle size(Table 3) with less variability. Figure 3 represents the response surface 3D plot and contour plots for particle size distribution data including Z-average, D_{10}, D_{50}, D_{90}.



(3a)



(3b)



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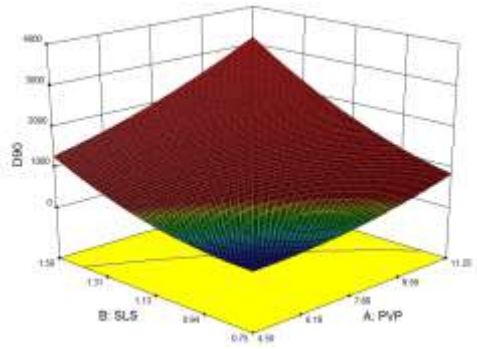


(3d)

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

Polydispersity Index

The combined effect of SLS and PVP on the PDI was studied using the response surface methodology. Figure 4 shows the response surface 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 the PVP concentration. The higher the concentration of PVP the narrower is particle size distribution resulting in a smaller PDI and the effect of PVP concentration on the PDI was observed to be independent of the SLS concentration. The factor SLS concentration showed only a limited influence on the PDI. Batch with higher PVP concentration has shown the least PDI value.

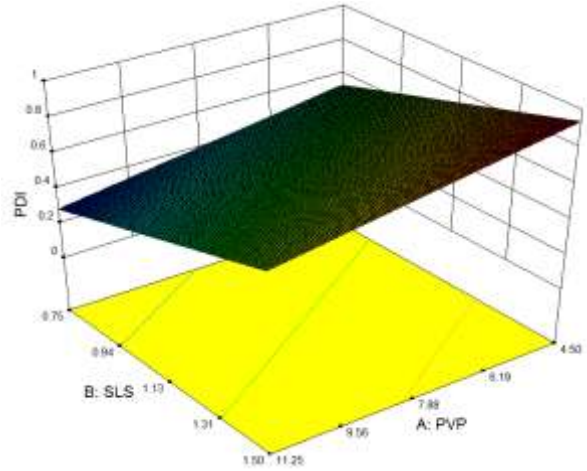


Figure 4: Response surface 3D plot and its contour plot showing effect of PVP and SLS on PDI

Zeta potential

From the Figure 5 it has been observed that the higher concentration of SLS yielded higher zeta potential thus with the increase in the concentration of the SLS the zeta potential also increased. With the increase in the concentration of PVP also there is increase in the zeta potential but the increase is not very significant. Thus the effect of the SLS on the zeta potential is independent of the PVP concentration.

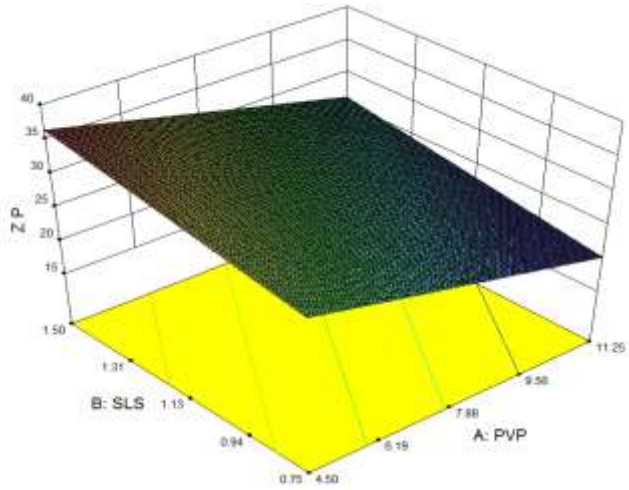


Figure 5: Response Surface 3D Plot Showing Effect of PVP and SLS on ZP

From the formulation optimization study QF-09E batch was found to be optimized composition having 5.25 % of PVP concentration and 0.75 % SLS concentration. Overlay plot was prepared using Design Expert 9.0.1 software. From the overlay plot particle size Z-average of 141 nm PDI 0.4 zeta potential 28 should come at the 5.74 % PVP concentration and 0.76 % SLS concentration. Figure 6 represents the overlay plot for the optimization of the composition.

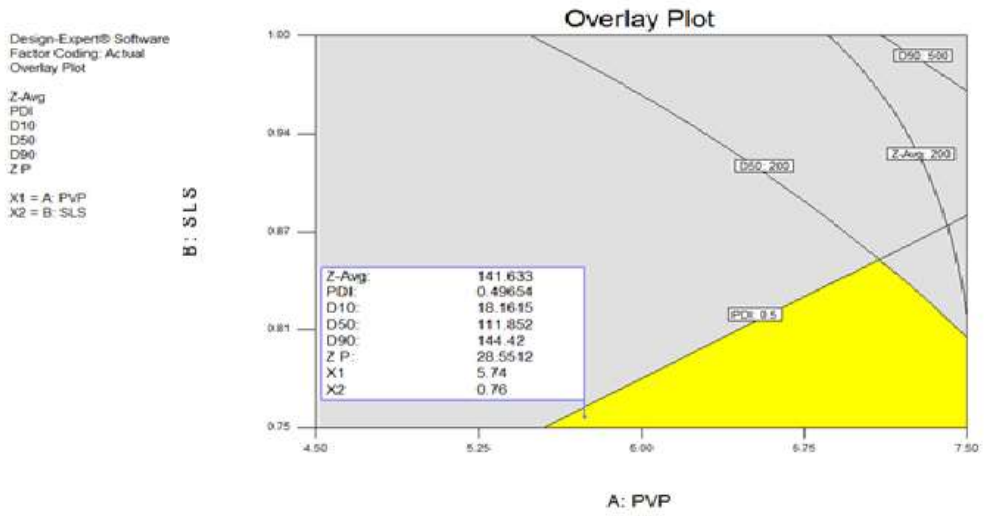


Figure 6: Overlay plot for composition optimization

Process optimization study

Nine compositions QF-08A to QF-08I were prepared and studied for process optimization. In all the batches the concentration of drug (5%), PVP (5.25 %) and SLS concentration (0.75 %) was kept constant. The data generated for PS, PDI and ZP was captured in table 4.

Table 4: Experimental Results of Process Optimization

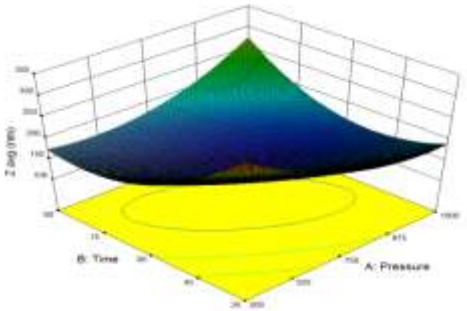
B. No	Z-average	Particle Size Distribution			PDI	ZP
		D10	D50	D90		
QF-08 A	345	34	215	281	0.7	42
QF-08 B	185	34	199	243	0.6	35
QF-08 C	181	36	174	215	0.3	39
QF-08 D	231	30	156	262	0.4	33
QF-08 E	133	27	185	244	0.5	30
QF-08 F	176	28	153	219	0.2	37
QF-08 G	184	27	165	219	0.5	24
QF-08 H	173	27	265	264	0.5	33
QF-08 I	275	29	280	244	0.6	35

To further analyze the effect of the variables on the responses, response surface 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.

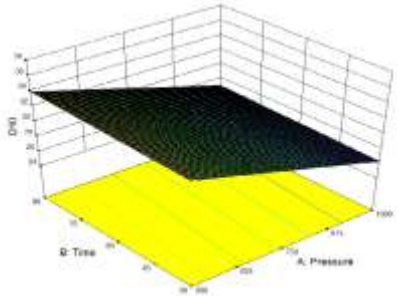
Particle Size

The effect of the homogenization time and pressure on the particle size has been studied and the response surface plots along with their contour plots have been captured in figure 7a to 7d. From response surface plot of Z-average it has been observed that at the low to medium (i.e 500 to 750 bar) homogenization pressure, with the increase in the homogenization time the particle size was

almost constant at initial and later stage of homogenization time with the slight reduction in the particle size at approximately 40 minutes of homogenization. However at higher pressure with the increase in the homogenization time there is increase in the particle size and shown high variability at 90 minutes of homogenization time period. With the RSM plot of D50 the same observation was made that at low pressure with the increase in the homogenization time period the particle size was slightly increased however at high pressure with the increase in homogenization time period the particle size also increased with high variability at 90 minutes of homogenization time. This variability was observed only at high pressure. With increase in pressure, the particle size D10 was almost constant but with increase in time the particle size also increased. With respect to D90, at low pressure the particle size was reducing with the increase in the homogenizing time and at high pressure particle size was almost constant. At initial time of homogenization, the particle size was slightly reduced with the increase in the pressure and at later time of homogenization the particle size was almost constant upto medium pressure and thereby increasing. Though the particle size at 750 bar and 60 minutes is less, the PDI was high and zeta potential was low. Taking all the dependent variables into consideration 750 bar pressure and 90 minutes of homogenizing time was chosen as optimum process conditions.



(7a)



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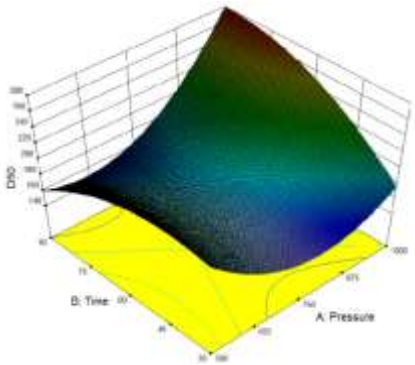

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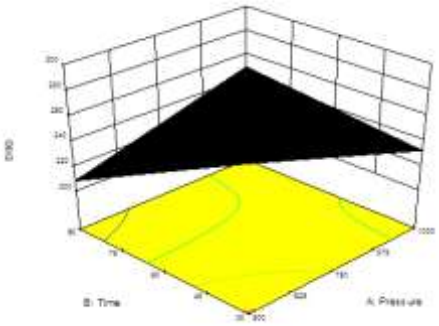


(7d)

Figure 7: Response surface 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).

Polydispersity Index

From the Figure 8 it has been observed that the Polydispersity index is affected by both homogenization pressure and time. At initial stages of homogenization ie. 30 minutes it has been observed that the PDI value decreased to certain extent and then again increased with the increase in the pressure. Hence low PDI value has been observed at medium pressure. And at later stages of homogenization ie at 90 minutes with the increase in the pressure from medium to high there was increase in the PDI value showing broader particle size distribution at higher pressure and also highly variable. The batch with 750 bar after 90 minutes of homogenization showed the least PDI value 0.2, owing to the narrower particle size distribution.

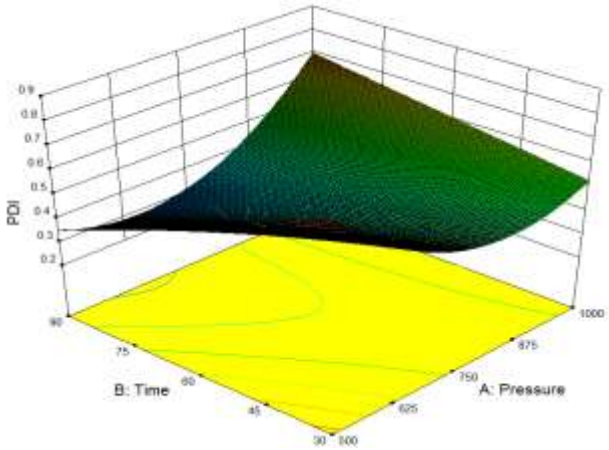


Figure 8: Response surface 3D plots showing effect of independent variables homogenization time and pressure on the dependent variable PDI

Zeta potential

From the Figure 9 it has been observed that at low pressure with the increase in the homogenization time, there is increase in the zeta potential. From the table 4 it has been observed that the highest zeta potential of 42.5 was observed at 500 bar after 30 minutes of homogenization but the PDI value was high 0.7 owing to its broader particle size distribution. For the batch with 750 bar after 90 minutes of homogenization the zeta potential was 37 and also the PDI value was minimum owing to its narrower particle size distribution. With the increase in the pressure the ZP reduced and is constant at 90 minutes. But at low homogenization time period the ZP was slightly reduced with increase in the pressure.

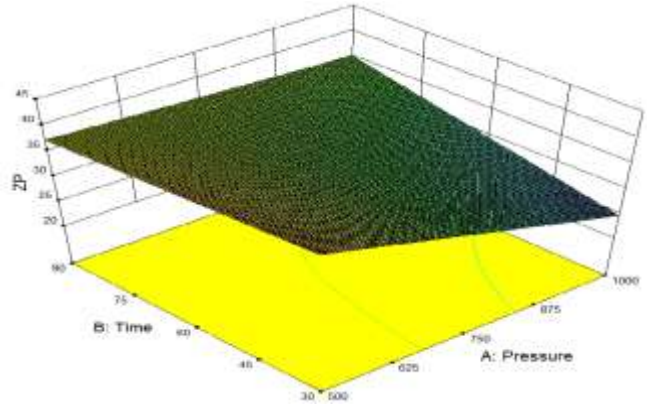


Figure 9: Response surface 3D plots showing effect of independent variables homogenization time and pressure on the dependent variable zeta potential

From the process optimization study QF-08F batch was found to be optimized compositionwith 750 bar and 90 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 Z-average of 176 nm; PDI 0.3; zeta potential 37 should come at 750 homogenization pressure and 88 minutes of homogenization. Figure 10 represents the overlay plot for the process optimization.

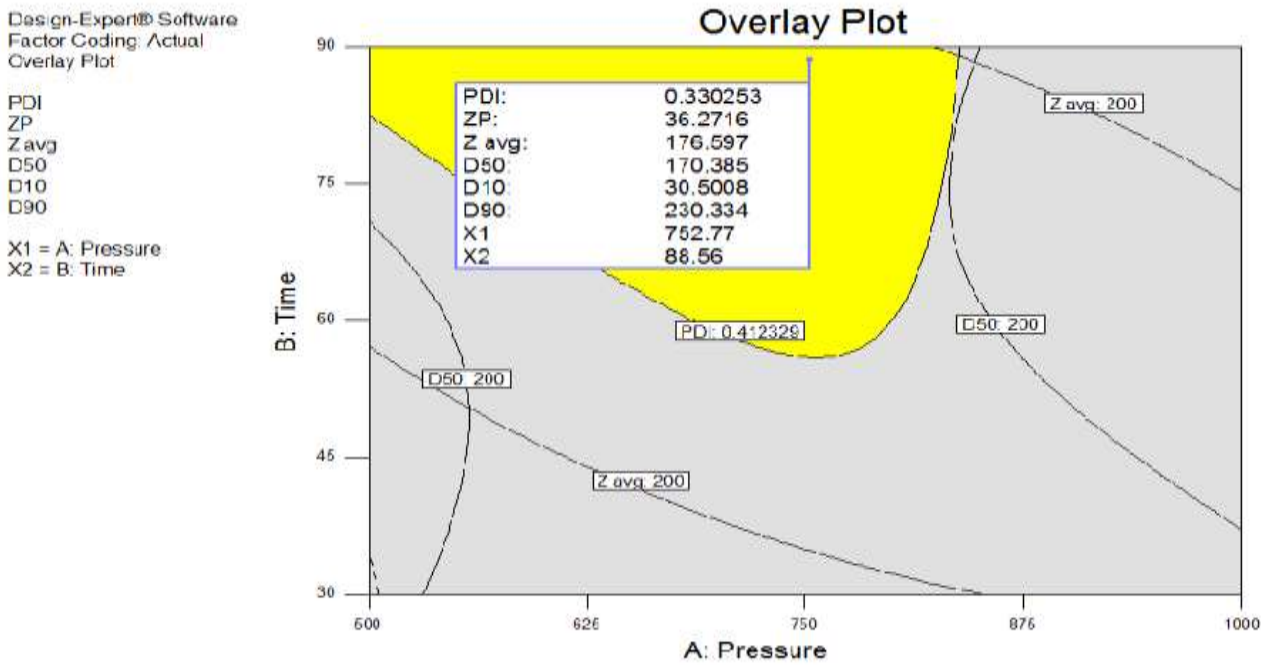


Figure 10: Overlay plot for the process optimization

Reproducibility and Stability

Nanosuspension with the optimized concentration of the PVP and SLS was prepared with optimized homogenization pressure and time as shown in table 5 at 1 liter 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.

Table 5: Optimized composition and process conditions

	Ingredients	Optimized parameters
Formulation	Quetiapine Fumarate	5 % w/w
	Polyvinyl pyrrolidone	5.25 % w/w
	Sodium lauryl sulfate	0.75 %w/w
Process	Homogenization pressure	750 bar
	Homogenization time	90 minutes.

Table 6: Data Generated for Optimized Batch

	Z-average	Particle Size Distribution			PDI	ZP
		D10	D50	D90		
Initial	131	22	128	192	0.3	35
1 month	139	19	132	226	0.4	33
2 month	123	28	149	262	0.2	37
3 month	140	34	153	219	0.2	36

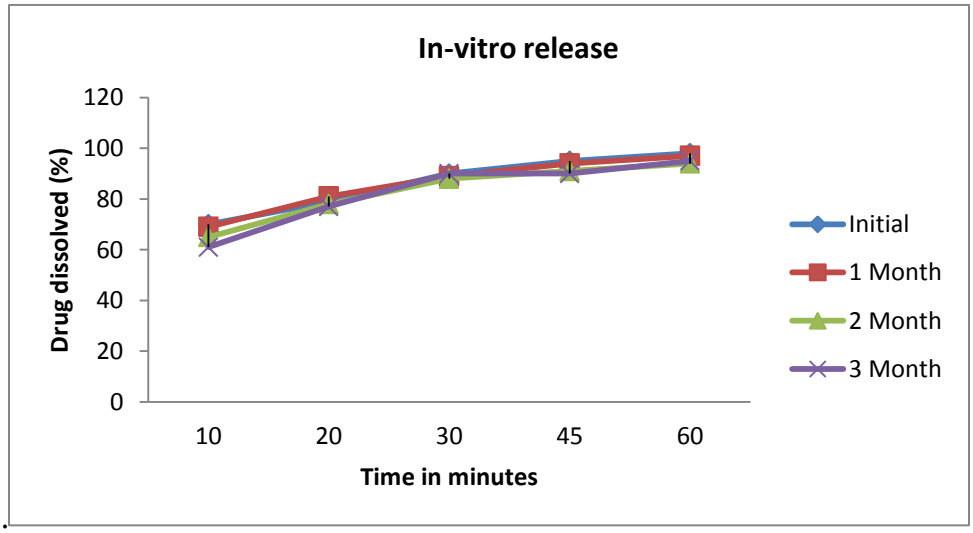


Figure 11: *In vitro* release profile of Optimized batch at initial and stability conditions

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 and 12b

represent the XRD of QF and spray dried nanoparticles. Figure 13a and 13b represent the DSC of QF and spray dried nanoparticles. Figure 14 represent the IR spectra of spray dried nanoparticles. Figure 15a and 15b represent the SEM photographs of QF and spray dried nanoparticles. From this data it has been observed that drug after reduction of the particle size to nano size the drug lost its crystalline nature. SEM photograph showed the spherical drug nanoparticles.

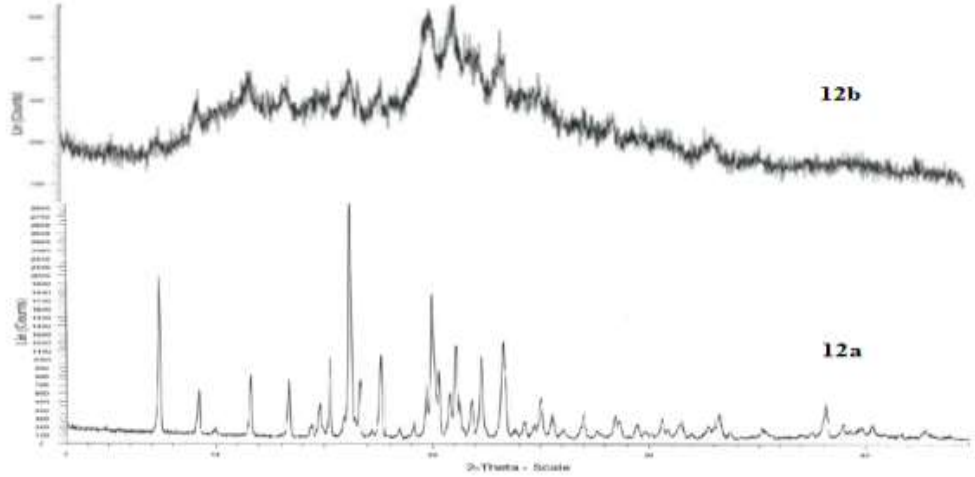


Figure 12: Shows the XRD of the QF (12a) and Spray Dried Powder of Optimized Nanosuspension

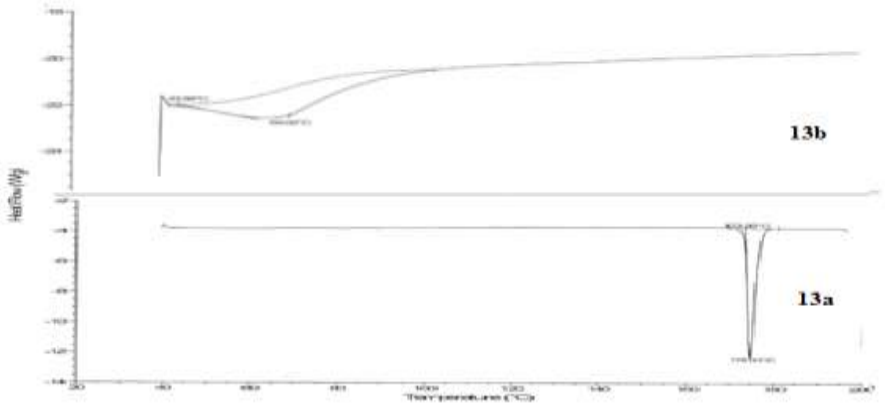


Figure 13: Shows DSC Plot of QF (13a) and Spray Dried Nanoparticles (13b)

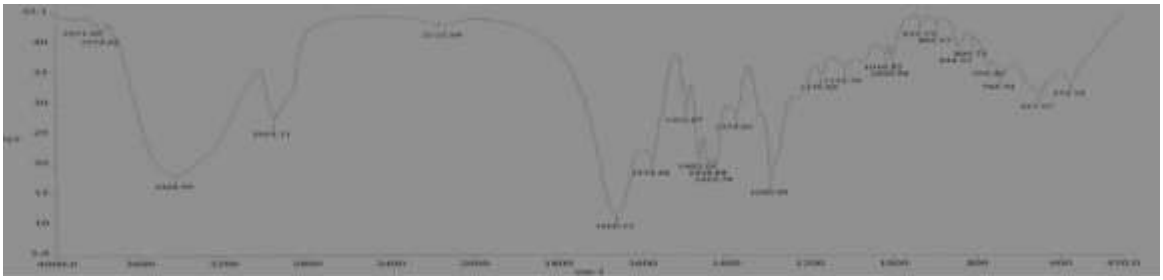
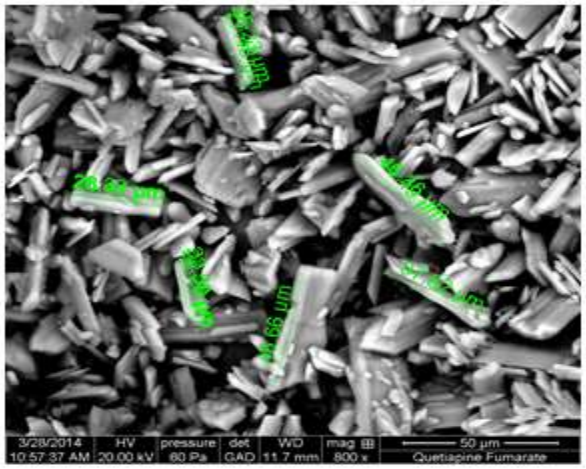
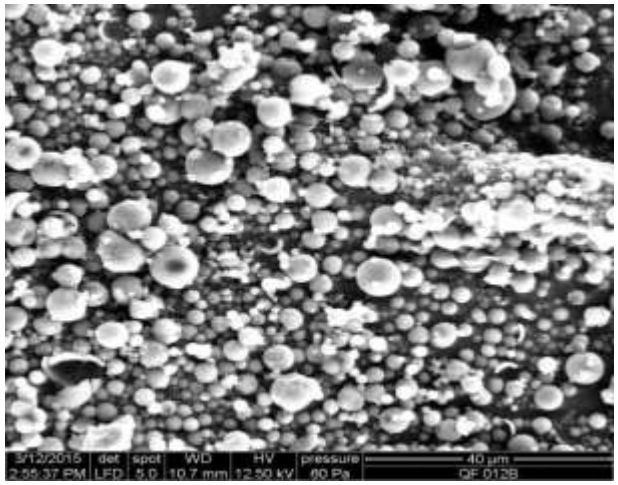


Figure 14: Shows the IR Spectra of the Spray Dried Powder of the Optimized Nanosuspension





15 (a) **15 (b)**

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

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 lower concentration of steric stabilizer i.e. PVP, the particle size is low however since the variability in the particle size data is high at lower concentrations, the medium concentrations were chosen as the optimum concentrations. The poly dispersity index was dependent solelyon the concentration of PVP and zeta potential was mainly dependent on the concentration of SLS. Similarly from the process optimization study it has been observed that at low and high pressure the particle size data was variable. At 90 minutes of homogenization time the particle size (D90) was observed to be constant at low to medium pressure and variable at high pressure. Zeta potential was variable at higher pressure and initial homogenization time period, however at 90 minutes of homogenization time period irrespective of pressure the zeta potential was observed to be constant. The loss of drug crystallinity after subjecting to spray drying process was confirmed by XRD and DSC analysis. SEM photograph of spray dried powder has shown the spherical nanoparticles. The drug nanoparticles have shown more than 80% of drug release in about 30

minutes. The prepared nanoparticles were found to be stable after 3 months of storage at room temperature.

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