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Comparative Evaluation of Lyophilization, Spray Drying and Spray Granulation for Converting Quetiapine Nanosuspension into Dry Powder

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Lakshmi Prasanna Gubbala*[1], Srinivas Arutla[2], Vobalaboina Venkateshwarlu[3]

[1]Integrated Product Development, Dr Reddys Laboratories Limited, Hyderabad. India.

[2]Head-Product Development; Apotex Research Private Limited, Bangalore. India.

[3]Managing Director, Neuheit pharma technologies Private Limited, Hyderabad, India.

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ABSTRACT

Objective: Objective of the current research work is to evaluate Lyophilization, spray-granulation (SG), spray-drying (SD) techniques and identify suitable method for converting the nanosuspension into dried form. **Methods**: Quetiapine fumarate nanosuspension was dried by Lyophilization, Spray granulation (SG), Spray drying (SD) and characterized for mean particle size (MPS), polydispersity index (PDI), zeta potential(ZP), X-ray diffraction (XRD), Differential scanning calorimetry(DSC), Fourier transform infra-red(FTIR), flow properties, moisture and drug content, saturation solubility and *in-vitro* release. **Results and Discussion**: The MPS of SG/SD nanoparticles was same as nanosuspension (165 nm) but lyophilization nanoparticles has 357 nm. SG/SD nanoparticles show narrow particle size distribution. ZP of the all nanoparticles was more than 25. The XRD/DSC shown loss of drugs crystallinity. SEM images showed change in morphology. The nanoparticles obtained by SG/SD/lyophilization increased the saturation solubility by 4, 5, 3 folds respectively and SG/SD nanoparticles dissolved > 70 % within 10 minutes whereas only 30 % of quetiapine dissolved. **Conclusions:** Out of the three processes evaluated SG and SD processes were promising method to produce the stable nanoparticles with enhanced flow properties, saturation solubility and dissolution rate that is attributed to a combination of amorphization and nanonization with increased surface area and improved wettability.

INTRODUCTION

Poorly soluble drugs face the problem of low oral bioavailability and erratic absorption due to their low dissolution velocity and saturation solubility (i). The use of poorly water-soluble drugs has a number of drawbacks, such as higher dose, higher administration frequency and the resultant occurrence of side effects (ii). In order to overcome poor solubility, numerous approaches have been developed. Among them formulation approach is considered a better avenue as it does not bring any changes in the chemical structure of the molecules and hence there is no alteration in pharmacological activity. The rate limiting step in the absorption process for poorly water- soluble drugs is the dissolution of such drugs in the gastrointestinal fluids rather than the rapidity of their diffusion across the gut wall. It is important to improve the oral bioavailability of these drugs by improving their dissolution rate and solubility. Various techniques used for the improvement of the dissolution rate of poorly water-soluble drugs include micronization, nanotechnology, formation of inclusion complexes with cyclodextrin, formation of amorphous drug and the formation of solid dispersions (SDs) with hydrophilic carriers (iii). Nanocrystals enhances absorption rate and oral bioavailability of hydrophobic drug molecules in the biological system (iv).

In the large field of nanotechnology, polymer based nanocomposites have become prominent area of current research and development (v). The reduction of particle size increases the particle surface area thereby the enhancement of solubility and dissolution. This enhancement insolubility can be explored on the possibility of reduction of dose in the high dose of poorly soluble drugs.Out of all the dosage forms, oral dosage forms are preferred. In order to prepare an effective solid dosage form from a drug nanosuspension, it is imperative that the nanosuspension is first dried and the dried nanoparticles should go back to their original particle size when reconstituted in an aqueous system (vi).

Nanosuspensions obtained after drug nanosizing results in an increase of dissolution profile and saturation solubility of hydrophobic molecules. It acts by decreasing the particle size of the hydrophobic molecules. They are a carrier free submicron colloidal drug delivery stabilizers. High pressure homogenization is used for drug nanosizing. This is simple fast and cost effective

reproducible method (vii) to reduce the size to nano-range and thus can help in reducing the desired dose thereby decreasing the side effects (viii).

Quetiapine fumarate (QF) is an atypical antipsychotic approved for the treatment of schizophrenia, bipolar disorder, and along with an antidepressant to treat major depressive disorder. QF is a white to off white powder, soluble in dimethylsulphoxide and poorly soluble in water. Quetiapine is having only 9% oral bioavailability (ix).

A nanosuspension not only solves the problems of poor solubility and bioavailability but also known to alter the pharmacokinetics of drug and thus improves drug safety and efficacy (x). Nanosuspensions can be defined as suspensions of nano-sized drug particles suspended in stabilizer/s solution/s. These solutions can be aqueous or non-aqueous and the typical size range for pharmaceutical nanosuspensions is 10-1000nm. The large surface area generated during the milling process leads to an increase of the free energy of the system. This increases the tendency of the system to revert to its lower energy state by agglomerating, thereby affecting the physical stability of the nanosuspension in the liquid form. Nanosuspensions being a liquid dosage form are physically and chemically unstable. Nanosuspensions formulations are known to undergo ostwald's ripening as well as phase separation or precipitation. To prevent chemical as well as physical instability, drying of nanosuspensions is employed. The advantages of dried formulations are improvement in chemical and physical stability and processibility into tablets or capsules. Drying can also create additional thermal stresses due to heat while spray drying or freezing for lyophilization that may destabize the particles. Morphology of the dried particles sometimes indicates the formation of irregular aggregates as well as donut shaped particles. The drying process itself can lead to issues such as aggregation on re-dispersion of dried powders, drug degradation and delayed dissolution rates. Due to these considerations identifying the suitable process and understanding the process and the critical parameters that have a strong impact on the redispersibility of the dry powders is important.

Proper selection of a stabilizer is also critical to overcoming physical stability issues associated with a nanosuspension formulation. The approaches commonly used to stabilize a nanosuspension formulation include steric stabilization or electrostatic stabilization or a combination of both (electrostatic stabilization). Steric stabilization is achieved by adsorbing

polymers onto the drug particle surface whereas electrostatic stabilization is obtained by adsorbing charged molecules, either ionic surfactants or charged polymers onto the particle surface (xi). Chemical stability issues (like hydrolysis of nanosuspension components and chemical reactivity of the drug during storage) are frequently encountered upon prolonged storage of nanosuspensions in the liquid form (xii). To mitigate these issues and to benefit from the advantages associated with a solid dosage form from the perspective of patient compliance, it is often essential to convert the nanosuspension into dry powder form that can be subsequently filled into capsules or compressed into a tablet.

Nanosuspension can be dried via conventional pharmaceutical drying operations, such as fluid bed coating/granulation and spray drying, lyophilization/freeze drying, vacuum drying, evaporation by heating, etc. Among the different methods for converting the liquid nanosuspension into dry nanoparticles are, spray drying, lyophilization and spray granulation are typically used to dry nanosuspensions (xiii, xiv, xv,xvi). The main challenge is in preserving the redispersibility of nanoparticles upon reconstitution with water or gastric fluids. Redispersants must be added to the nanosuspensions prior to the drying step. Common redispersants are sugars such as sucrose, lactose, and mannitol similar to those used for freeze drying or lyophilization. Generally redispersibility depends primarily on the choice of redispersants, but sometimes depends on the choice of both the polymeric and surfactant stabilizers.

Aggregates formed during the drying should rapidly disperse so that the original particle size is regained within a short period of time. For oral formulations, the transit time of the particles in the gastrointestinal tract typically ranges from < 1 to 8 hrs, depending on the region of absorption. Hence particle dissolution should occur in a short time frame (on the order of minutes) to facilitate availability of the drug for absorption. Variation in disintegration and dissolution times due to the presence of aggregates can cause unpredictable variations in bioavailability.

Freeze drying has been widely used to convert nanosuspensions into solid forms with a comprehensive evaluation of formulation and processing parameters reported in the literature (xvii). Spray drying is another popular approach widely used (xviii). An alternative to these two processes for drying of nanosuspension would be spray granulation based approach (xix).Additional excipients may need to be added to the powder to improve further processibility.

The main purpose of this research work is to compare and evaluate the three different drying techniques for drying the nanosuspension and choose suitable method for obtaining stable dried nanoparticles.

MATERIALS AND METHODS

Materials:

Quetiapine Fumarate was obtained from Dr Reddys Laboratories limited. Polyvinyl pyrrolidone [PVP] supplied by BASF, sodium lauryl sulfate [SLS] by JRS, Mannitol by Rouquette Pharma, Lactose by Meggle pharma. Lyophilizer, Spray drier, Fluid bed coater/Granulator, High pressure homogenizer are the equipment used for manufacturing nanosuspension and drying. All other solvents and reagents used were of HPLC or analytical grade.

Preparation of nanosuspension

In this work, high pressure homogenization process was used. 3L batch size of nanosuspension was prepared by dissolving polyvinyl pyrrolidone (5% w/w) and sodium lauryl sulphate (0.75% w/w) in an aqueous solution into which the drug Quetiapine fumarate (5% w/v) was dispersed. The resulting suspension was stirred for about 15 minutes followed by high shear homogenization at 4000 rpm for about 15 minutes to uniformly disperse the drug. The suspension was then subjected to high pressure homogenization at 500 bar for about 2 cycles (5 minutes) followed by 750 bar pressure for about 90 minutes (15 cycles).

Production of dried nanoparticles

Drying of nanosuspensions can cause destabilization of the particles; leading to irreversible aggregation hence it is very important to screen different methods to identify suitable method to produce stable dried nanoparticles. The nanosuspension prepared as above was divided into 3 equal portions of 1000 ml each and each portion is subjected to drying. Before subjecting the nanosuspension for freeze drying or spray drying 10 % bulking agent or re-dispersant such as mannitol or lactose are added to the nanosuspension.For spray granulation mannitol or lactose was used as substrate on which the nanosuspension was sprayed. During drying care has been taken that no aggregates are formed.

Freeze drying

The principle of freeze drying method is that small amounts of a product will be frozen in and thereafter it will be placed under vacuum. Through vacuum the frozen liquid sublimates. The ice immediately changes into vapour, without to defrost first, also called sublimate. During process, the outside part will be the first part that dewaters. After that, the water is removed closer and closer till the core of the product. Hereby the structure of the product stays intact. Due to the vacuum the ice will evaporate immediately without turning into water again. The freeze dried products is usually of high quality, mainly because the temperature stays low during the whole process.To 150 ml of above prepared nanosuspension added 15 g of mannitol (QF-010A) or 15 g of lactose (QF-010B) with stirring. Then the nanosuspension was subjected to lyophilization. The lyophilization cycle includes overnight freezing (for about 20 hours) at -60 °C, 150 mTorr vacuum following by drying for about 24 hours at 120 mTorr vacuum conditions.

Spray granulation

Spray granulation of the nanosuspension was performed using GPCG-1.1; FR463; PtamGlatt fluid bed coater. The fluid bed dryer was preheated prior to the start of the spraying process. Sufficient fluidization of the substrate (mannitol/lactose) was maintained to ensure that there was no clumping or agglomeration of powder during the granulation process. Spray granulation was carried out wherein, in 1.1 liter fluid bed granulator or coater (GPCG-1.1; FR463; PtamGlattmannitol (QF-011A) or lactose (QF-011B) was taken and spray granulated (bottom spray) by using nanosuspension. The following are the conditions used for spray granulation

Inlet temperature: 70^0C
Product temperature: 38 ^{0}C
Blower drive speed: 15
Atomizing pressure: .0.8 bars
Spray pump speed: 2-5
Spray rate: 0.07 g/minute.

Spray Drying:

This method isn't really old yet and actually the most remarkable method which is about drying of liquids which can't be heated through their properties without a significant change of the product properties. Basically, the principle works like this, liquid or substance will be sprinkled into a hot cylinder by a sprayer. During the downfall, the liquid will evaporate. That liquid will lift off and will be captured. The product that falls down is solid powder which is separated from the air by a cyclone collector. In spray dryers, the material that has to be dried will suspend in the air that is to say that the liquid changes into a misty fog (atomized) which has a large surface area. The atomized liquid will be exposed to a steam hot air in a drying room. The liquid evaporates fast and the solids recover as powder that exists of fine, hollow spherical parts.

To 1000 ml of the nanosuspension, 10% of mannitol (QF-012A) or 10 % of lactose (QF-012B) was added with stirring until uniform suspension was obtained. The resultant suspension was spray dried using Buchi mini spray dryer B-290 with inlet temperature 140 °C, inlet nitrogen pressure 5 kg/cm2and liquid suspension feed rate 6 ml/minute.

Characterization of the nanosuspension and dried nanoparticles

The nanosuspension obtained was characterized for particle size, zeta potential and polydispersity index. The dried nanoparticles are characterized for particles size, zeta potential, polydispersity index, X-ray powder diffraction, differential scanning calorimeter, Fourier transform infra-red spectroscopy, Scanning electron microscopy, flow properties, moisture content, drug content, saturation solubility, dissolution profile.

Particle size and polydispersity Index [PDI]

The particle size, which is represented in terms of d10, d50, d90 affects the solubility of the poorly soluble drug. The particle size distribution and its range named polydispersity index (PDI) can be determined by laser diffraction (LD), photon correlation spectroscopy, microscope, and coulter counter [xx].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 [xxi].The mean particle size (d50, ie 50 % of particles are having size less than this value) of the

nanosuspension before drying was estimated in triplicate by using Zeta sizer- Nano ZS, Malvern Instruments UK) at room temperature. A refractive index of 1.65 was used for particle size analysis. Nanosuspension was added to the sample dispersion unit (deionized water) and stirred at 2000 rpm to reduce the interparticulate aggregation and laser obscuration range was maintained between 10-20 %. The samples were adequately diluted with deionized water and placed in electrophoretic cell and measurement was carried out with help of software. The particle size was measured after performing the experiments in triplicates.

The particle size of drug in dried nanoparticles was analyzed by adding water to dried nanoparticles (so that the surface stabilizers, re-dispersants are in dissolved state and only drug in dispersed form) followed by dilution with water to obtain suitable concentrations for measurement in the same manner as carried out for the nanosuspension.

Zeta Potential

A prerequisite to achieve an enhancement of oral bioavailability with drug nanocrystals is that crystals 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 and a minimum of ±20 mV for steric stabilization. The zeta potential values are commonly calculated by determining the particle's electrophoretic mobility and then converting the electrophoretic mobility to the zeta potential.

Zeta potential of the nanosuspension has been analyzed in Malvern zeta sizer after diluting nanosuspension with water to obtain suitable concentration for measurement. Further, the zeta potential of the dried nanoparticles is obtained by adding water to the dried nanoparticles and diluted with water to obtain suitable concentration for measurement. The diluted sample was added in specialized zeta cell and the same procedure as that of particle size was carried out.

X-ray Powder diffraction (XRD)

X-ray powder diffraction measurements were carried out on samples using a diffractometer (X'Pert MPD Model, Phillips, Holland). The results were recorded over a range of 0–40^0 (2θ) using the Cu-target X-ray tube and Xe-filled detector. The operating conditions were: voltage 40 kV; current 30 mA; scanning speed 1/min. The dried nanoparticles obtained by lyophilization, spray granulation and spray drying has been characterized by XRD to study their morphological changes.

Differential Scanning Calorimetry (DSC)

DSC scans of the Quetiapine fumarate drug, the physical mixture of quetiapine, PVP, SLS and mannitol/lactose and dried nanoparticles obtained by lyophilization, spray granulation, spray drying,have been studied using DSC- Shimadzu 60 with TDA trend line software. All samples were weighed (8-10 mg) and heated at a scanning rate of 20^0C/min under dry air flow (100 ml/min) between 25^0 C and 220^0 at 10^0C/minute.

Fourier Transform Infra-Red (FTIR) Spectroscopy

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. Thus, the spectral interpretations should not be confined to one or two bands only actually the whole spectrum should be examined [30,31]. The pure drug quetiapine, individual excipients (PVP/SLS/mannitol/lactose); physical mixture of quetiapine, PVP, SLS and mannitol/lactose and dried nanoparticles obtained by lyophilization, spray granulation, spray drying have been studied on the sample prepared in potassium Bromide (KBr) disks, using Shimadzu Fourier Transform Infra-Red spectrometer. The powder blends for IR spectra was prepared by blending sample with KBr in 1:100 ratio and were scanned over wave number range of 400 to 4000 cm^{-1}.

Scanning Electron Microscopy [SEM]:

The morphology of the raw quetiapine fumarate, nanosuspension and nanosized quetiapine was examined with the scanning electron microscopy, operated at the low vacuum with a LFD detector and 60 Pa pressure. First, double sided carbon tape was stuck onto the clean aluminum stub. Then the freshly prepared nanosuspension or dried nanoparticles was applied on the carbon tape and excess was tapped off using nitrogen gas. 5nm thick gold coating was applied onto the sample. The dried nanoparticles obtained by the different drying methods such as lyophilization, spray granulation and spray drying using two different dispersants has been characterized by SEM analysis for studying the morphology of the dried nanoparticles.

Flow properties, moisture and drug content of the dried nanoparticles.

To determine the flow properties of the dried nanoparticles, four parameters such as angle of repose, bulk density, tapped density, Carr's Index and Hausner ratio have been determined for all the six batches. The angle of repose was determined by allowing dried nanoparticles to flow through the funnel which is fixed to the stand at a height of approximately 2 cm from the surface on a plane surface. The flow was continued till the pile formed touches the stem tip of the funnel. Height of the pile was determined and bottom surface of the pile which is roughly circular shape is drawn and the radius of the pile was measured. Angle of repose was then calculated by using the following equation (I)

$$\tan \theta = h / r \qquad (I)$$

Where, θ = angle of repose

h = height of the pile

r = average radius of the powder cone

If the value of the angle of repose is less than 25 then it shows excellent flow and if the value is between 25-30 it shows good flow and if the value is more than 30 it indicates that the dried nanoparticles have poor flow properties.

The bulk density of dried nanoparticles was determined by placing approximately 25 g of the dried nanoparticles into graduated measuring cylinder and the volume occupied by the powder method was recorded. The tapped density of the dried nanoparticles was determined by

measuring the volume occupied by the dried nanoparticles after tapping continuously until there is no further change in the volume.

The Carr's Index (CI) value gives an indication of powder flow; it is determined by the following equation (II). If the Carr's Index value is less than 25% indicates a good flow whereas, a value greater than 25% indicates a poor flow.

$$\text{Carr's Index (\%)} = \frac{\text{Tapped density - Bulk density X 100}}{\text{Tapped density}} \quad \text{(II)}$$

Hausner ratio was calculated from tapped **a**nd bulk density using the following equation (III).A Hausner ratio value less than 1.20 is indicative of good flow whereas, a value > 1.5 indicates poor flow.

$$\text{Hausner Ratio} = \frac{\text{Tapped density}}{\text{Bulk density}} \quad \text{(III)}$$

Moisture content

The moisture content (expressed as loss on drying) of the dried nanoparticles has been determined by using halogen moisture analyzer. About 1 g of the dried nanoparticles has been spread on the tray in the sample holder and the powder sample has been heated to about 105 ^{0}C and the loss of water upon drying was obtained.

Drug content

The drug content of the nanosuspension and dried nanoparticles at initial conditions was estimated to know if there is drug degradation happened during drying process and also to study the impact of the drying methods on the drug content. Further, the dried nanoparticles by three different processes were estimated for drug content after storing at room temperature (RT) for 3 months. The drug content was determined by high pressure liquid chromatography (HPLC) method. A portion of nanosuspension or dried nanoparticles equivalent to 25 mg of quetiapine was taken in volumetric flask and dissolved in diluents (water: acetonitrile in 20:80 ratio) with sonication followed by centrifugation at 3000 rpm for about 10 minutes. The solution was

filtered through 0.45 µm Durapore PVDF membrane filter and was analyzed by HPLC. The mobile phase consists of mixture of potassium dihydrogen orthophosphate, acetonitrile, tetrahydrofuran, triethylamine pH adjusted to 6.4 with KOH solution. Chromatographic separation was accomplished using an Xterra Column RP8 3.5 µm; 4.6X150 mm column. The mobile phase was pumped isocratically at a flow rate of 1.5 ml/minute during analysis and maintained at a column temperature of 50 °C and detection wavelength of 217 nm.

Saturation Solubility

Saturation solubility is a compound-specific constant only depending on the temperature and the properties of the dissolution medium. However, below a size of approximately 1–2µm, the saturation solubility is also a function of the particle size. Saturation solubility of plain drug (Quetiapine Fumarate and dried nanoparticle formulations obtained by lyophilization, spray granulation, spray drying were carried out in 0.1 N HCl, 4.5 acetate buffer, 6.8 phosphate buffer and purified water. Excess amount of the drug or dried nanoparticles have been added to 100 ml of media maintained at 37^0C and shaken on rotary shake flask for a period of 24 hours. The samples were taken into centrifuge tube and centrifuged for about 10 minutes at 4000 rpm. The supernatant was collected and filtered through 0.22 µm nylon membrane filter, diluted with diluents (methanol and acetonitrile in 1:1 ratio) and analyzed using HPLC (waters Alliance HPLC system USA) method same as that used for determination of drug content.

***In-Vitro* Dissolution Study:**

The dissolution profiles of plain drug quetiapine fumarate and dried nanoparticles was determined in a USP apparatus II in 900ml phosphate buffer pH 6.8. The dissolution media was maintained at 37±0.5^{0}C with a paddle rotation speed at 50 rpm. The amount of drug used was equivalent to 25 mg. Dried nanoparticles equivalent to 25 mg was taken for analysis. At specified time intervals (10, 20, 30,45, 60 minutes) 5ml of dissolution media were withdrawn and replaced with an equal volume of the fresh medium to maintained at 37^0C to maintain a constant total volume. Samples were filtered through a 0.22µm nylon membrane filter (Millipore, Bedford, MA) and assayed for drug content in the same manner as carried out for drug content.

RESULTS

Mean Particle Size [MPS]

The method selected for the preparation of nanosuspension was high pressure homogenization and sodium lauryl sulphate, polyvinylpyrrolidone as surface stabilizers. As can be seen from Table 1, there was a vast difference in the particle size distribution of the raw drug and the homogenized nanosuspension. Also, a smaller particle size and narrower PSD of Quetiapine nanoparticles were obtained. Further, the reproducibility of particle size of nanosuspension as well as dried nanoparticles was obtained (Table 1), which should be more beneficial to enhance the stability of Quetiapine nanoparticles. The dry powder with nano-sized Quetiapine exhibited good uniformity. Additionally, the nanosuspension and dried nanoparticles obtained were stored at room temperature (RT)(such as temperature about 20^0C to 25 ^{0}C)for three months and characterized for mean particle size (MPS), polydispersity index (PDI) and zeta potential (ZP).

From table 1 it has been observed that the MPS obtained for nanosuspension obtained through high pressure homogenization for 90 minutes is 165 nm. The mean particle size of the nanosuspension after storage at RT for 3 M is 185 nm. Further, the MPS of dried nanoparticles obtained by lyophilization, spray granulation and spray drying is 357 nm, 185 and 154 nm respectively. MPS for nanoparticles obtained by lyophilization is high when compared to spray granulation or spray drying. This trend has been observed in the mean particle size obtained for the dried nanoparticles stored at room temperature for 3 months. The MPS of dried nanoparticles stored at RT for 3 M obtained by lyophilization, spray granulation, spray drying is observed to be 465nm, 195nm, 160 nm respectively.

Polydispersity Index [PDI]

The PDI of the initial drug suspension, nanosuspension and the dried nanoparticles obtained by three different processes has been captured in table 1. The PDI of the initial drug suspension before subjecting to homogenization was observed to be 0.8 indicating broad particle size distributions whereas after preparing the nanosuspension the PDI observed to be 0.233 indicating the narrow particle size distribution. Further from the PDI of the nanoparticles, it has been observed that the PDI for lyophilized nanoparticles is broad when compared to that obtained by

spray granulation or spray drying. Further, the PDI of the dried nanoparticles obtained by lyophilization when stored at RT for 3 M has been increased to 0.781 indicating there is aggregation of the particles on storage leading to the broad range of particle size distribution.

Zeta potential [ZP]

The ZP of the drug suspension after homogenization has increased from 28 to 34 and maintained the same after storing at RT for 3M, indicating the stable nanosuspension. The ZP of the dried nanoparticles at initial as well as storing at RT for 3M has been observed to be more than 25 indicating the stability of the dried nanoparticles obtained by three different processes.

From the MPS, PDI and ZP data of the dried nanoparticles it has been observed that there is no obvious increase in parameters when dried by SG and SD when compared to that of lyophilization. This suggests the dried nanoparticles obtained by SG or SD are stable when compared to that obtained by lyophilization. However, it can be contributed that stabilization of the nanosuspension was linked to absorption of PVP on the surface of Quetiapine nanocrystals. From the previous optimization studies, it has been concluded that a combination of PVP and SLS (5:1, w/w) was the most successful of all the stabilizing agents investigated as far as the formation of stable Quetiapine nanosuspensions were concerned. The results indicated that PVP and SLS played a key role in inhibiting the growth of Quetiapine nanoparticles when dried by using SG or SD process.

Table 1: Particle size, PDI, ZP of raw Quetiapine Fumarate, nanosuspension and dried nanoparticles (each number represents mean ± standard deviation, n=3)

Samples	Particle size (µm)			PDI*	ZP#
	d10	d50	d90		
Quetiapine Fumarate	15± 4	49±7.2	104±9.5	0.813±0.16	28±3
Initial conditions					
Nanosuspension	0.131±0.009	0.165±0.011	0.198±0.005	0.233±0.1	34.1±2.4
Dried nanoparticles (lyophilization)	0.290±0.06	0.357± 15	0.444± 24	0.525± 0.34	35.6±3
Dried nanoparticles (SG**)SG	0.011± 0.004	0.185±0.006	0.261± 0.013	0.309±0.04	39.7± 3
Dried nanoparticles obtained by SD$	0.019± 0.002	0.154± 0.003	0.190±0.01	0.249±0.06	42.5±2
After storing at room temperature (RT) for 3 months					
Nanosuspension after	0.067±0.009	0.185±0.011	0.344±0.034	0.664±0.31	33.2±5
Dried nanoparticles (lyophilization)	0.387±0.029	0.465±0.18	0.562±0.24	0.781±0.19	27.5±6
Dried nanoparticles (SG)	0.170±0.005	0.195±0.002	0.219±0.014	0.332±0.005	32.8±2
Dried nanoparticles (SD)	0.010±0.003	0.160±0.008	0.229±0.021	0.314±0.008	34.5±2.4

* Polydispersity Index

Zeta potential (-mV)

**Spray granulation

$ Spray drying

X-Ray Diffraction

During the preparation of nanosuspensions high pressure homogenization was used and converting into dried nanoparticles by three different processes such as lyophilization, spray granulation and spray drying was employed. According to many reports, they all have the possibilities to decrease the degree of crystallinity of drug compounds or transform the drug crystals to its amorphous form [xxii]. Therefore, it is significant to investigate the effect of the above processes on the physical state of Quetiapine. The XRD patterns obtained of raw Quetiapine and dried nanoparticles obtained by lyophilization, spray granulation and spray drying is displayed in Figure 1. The raw Quetiapine is crystalline and exhibited crystalline peaks 2θ values at 7.4, 16.26, 20.1, 23.29& 38.23 from 10 to 30^0, indicating crystalline nature of

Quetiapine Fumarate. It has been observed that the characteristic crystalline peaks of quetiapine fumarate disappeared in the pattern of prepared nanoparticles revealing that the crystallinity of Quetiapine was decreased dramatically.The peaks observed in the XRD pattern of the formulation has been observed to be that of mannitol or lactose.

DSC Analysis:

In order to further confirm the physical state of nanoparticles in the suspension and in the dried nanoparticles, DSC analysis was performed. The DSC plots obtained on raw quetiapine fumarate, Quetiapine/PVP/SLS/Mannitol or lactose monohydrate physical mixture (physical mixture with mannitol comprise Quetiapine/PVP/SLS/Mannitol and similarly the term physical mixture with lactose comprise Quetiapine/PVP/SLS/lactose monohydrate) and dried nanoparticles obtained by three different processes have been represented in Figure 2 and Figure 3.

The DSC curve of raw Quetiapine fumarate showed a single sharp endotherm at 176 ^{0}C which corresponded to its melting point. The DSC curve of mannitol, lactose monohydrate showed endotherm at about 169-171^{0}C [22] and 200-22^{0}C [xxiii] respectively and broad melting endotherm at approximately 75 ^{0}C for PVP. For lactose monohydrate, an additional endotherm has been observed to be at around 140^{0}C corresponding to the monohydrate component of lactose which is in accordance to value disclosed in the reference Listiohaldi et al [xxiv]. The physical mixture showed the broad endotherms corresponding to that of drug quetiapine fumarate, polyvinyl pyrrolidone and mannitol/lactose. There is slight shift in the endotherm of the components in the physical mixture when compared to that of individual peaks.

Figure 3 shows the DSC curves of the dried nanoparticles prepared by lyophilization, spray granulation and spray drying. It has been observed that the characteristic endotherm peak of the quetiapine fumarate disappeared after drying process. In the curves the endotherm related to polyvinyl pyrrolidones, mannitol/lactose only observed. This further confirms the XRD interpretation that irrespective of any of the drying methods used, quetiapine fumarate lost its crystallinity.

FTIR spectroscopy:

Infrared (IR) provide structural information on a molecular level. The spectra are based on unique molecular vibrations that occur within a compound; hence the different fundamental molecular vibrations of differing polymorphic forms will give each its own unique fingerprint spectrum from which it may be identified. Any change in the structure of the drug quetiapine fumarate due to interaction between quetiapine fumarate and excipients such as PVP, SLS, mannitol, lactose monohydrate has been studied

From figure 4 the characteristic peaks of quetiapine fumarate observed at 3310 cm^{-1}may be due to O-H stretching, 3080 cm^{-1} may be due to Ar-H stretching; 2880 cm^{-1} due to C-H stretching, 1600 cm^{-1} may be due to C-N, 1597, N-H bending;1340 cm^{-1} may be due to C-H; bending, 1070 cm^{-1} C-C stretching, 1030 cm^{-1} may be due to –C-O-C group, 791 cm^{-1} may be due to substituted benzene ring; The peaks of 1647, 1463 and 1294 $cm^{-1}$are assigned to the C=O stretching, CH_2 bending and C-N stretching vibration, respectively in the PVP. The absorption peaks at 1407 cm^{-1} may be attributed to -S-O stretching of sodium lauryl sulphate, prominent peaks of mannitol has been observed at 3279, 2359, 2059 and 1420 cm^{-1}.

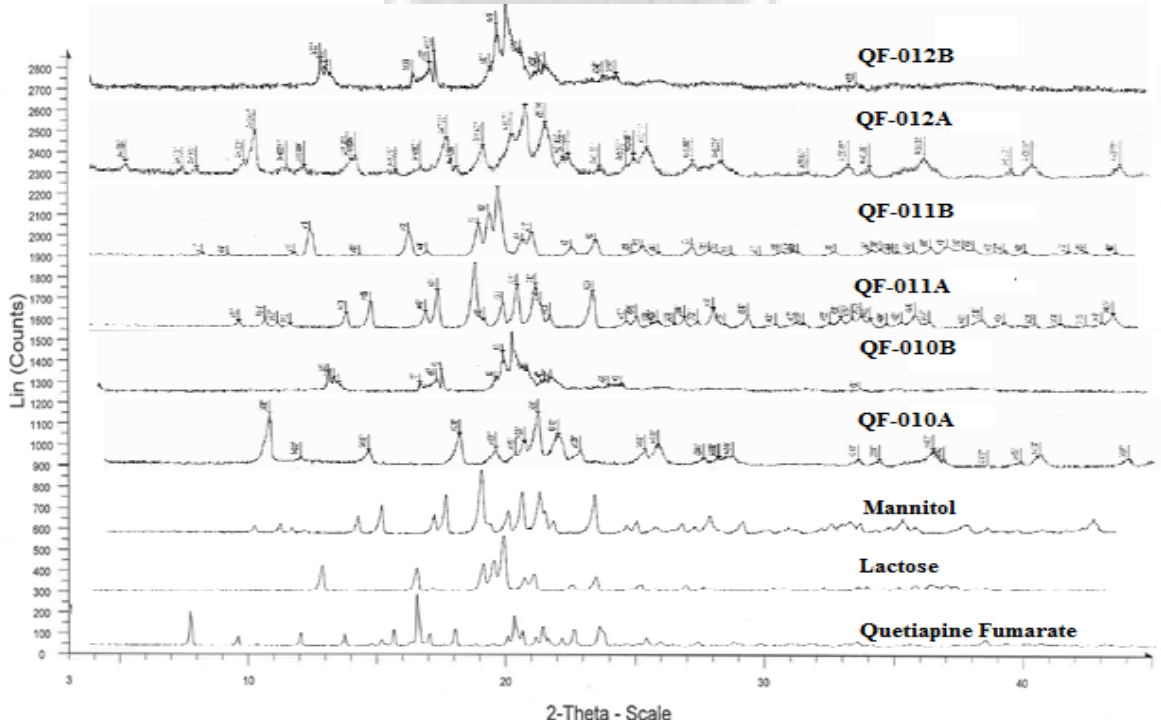


Figure 1: XRPD patterns of Quetiapine fumarate, Mannitol, Lactose monohydrate, QF-010A, QF-010B, QF-011A, QF-011B, QF-012A, QF-012B.

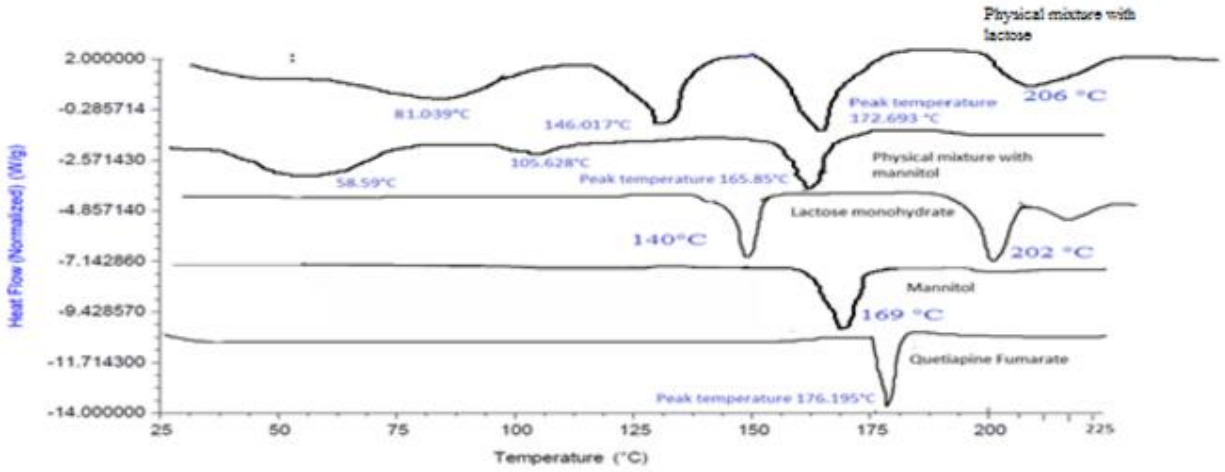


Figure 2: DSC curves of Quetiapine fumarate, Mannitol, Lactose monohydrate, Physical mixture with lactose, Physical mixture with mannitol.

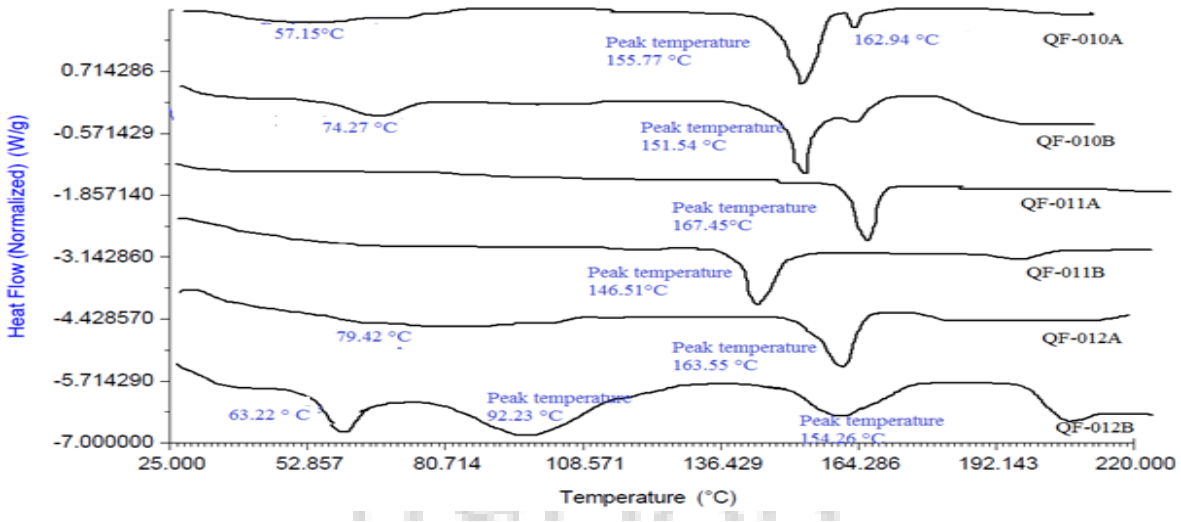


Figure 3: DSC curves of QF-010A, QF-010B, QF-011A, QF-011B, QF012A, QF-012B.

The monohydrate form exhibits a sharp, distinct, O_H stretch peaks at 3521.5/cm. The shape and location of these peaks are indicative of constrained water in the crystal lattice. Dehydration of the monohydrate form results in the disappearance of the peak at 3521.5/cm producing spectral evidence indicating water was removed from crystal lattice. From the IR spectrums of the QF, individual excipients, physical mixture and the dried nanoparticles, it has been observed that there is no appreciable change in the positions of the characteristic bands of the drug either in physical mixture or during drying by any of the three processes. Since there is no change in the

nature and position of the bands in the formulation, it can be concluded that the drug maintains its identity without going any chemical interaction with the surface stabilizer used.

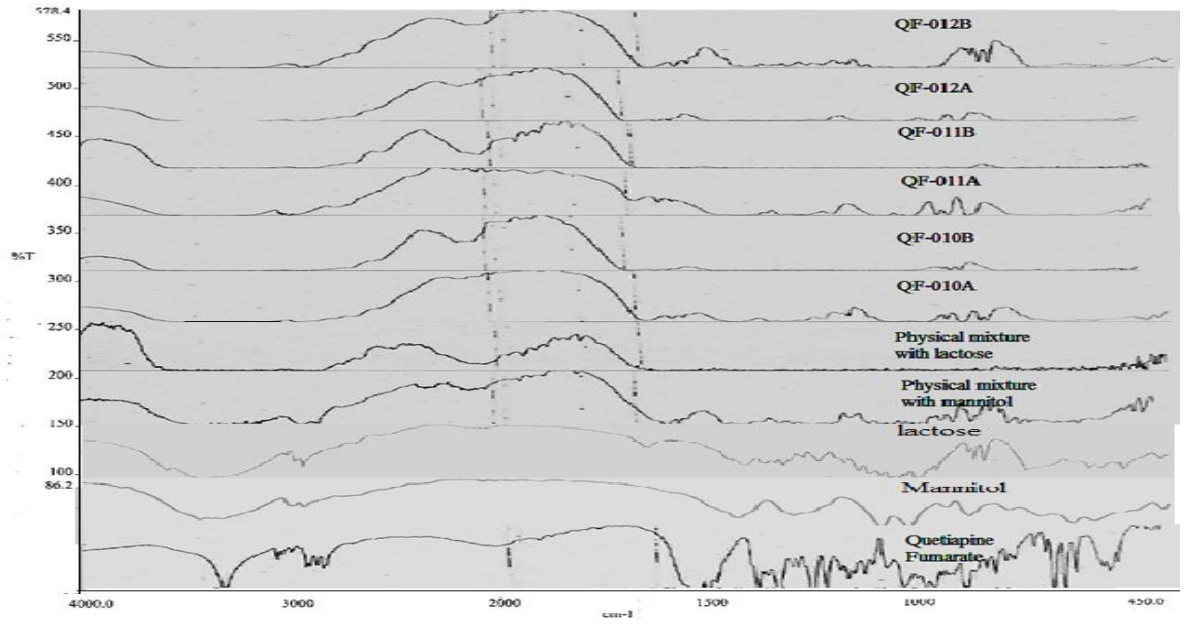


Figure 4: Infra-red spectra of Quetiapine fumarate, QF-010A, QF-010B, QF-011A, QF-011B, QF012A, QF-012B, lactose monohydrate, mannitol and their physical mixtures.

Scanning electron microscopy

Pure drug quetiapine fumarate, nanosuspension and its dried nanoparticles prepared by lyophilization, spray granulation and spray drying were been analyzed for surface appearance, particle size and shape by scanning electron microscopy. Pure quetiapine fumarate showed crystalline particles with particle size ranging from 12 to 44 microns. SEM photograph of quetiapine fumarate at higher magnification (A) and lower magnification are shown in Figure 5. It can be seen that the raw drug particles existed as predominantly needle shaped crystals.The SEM images of the nanosuspension before subjecting to the drying process has been shown in Figure 6 which clearly indicated how the needle shaped particles have been converted to spherical particles after subjecting to particle size reduction by high pressure homogenization.The SEM images of lyophilized nanoparticles prepared using mannitol as bulking agent are shown in figure 7 and figure 8 respectively. The SEM images with mannitol as bulking agent show pointed structures along with aggregates and again a large size range existed between the largest and the smallest particles whereas the SEM images with lactose as bulking

agent shows large chunks of random shaped particles having small particles stacked on top of them.

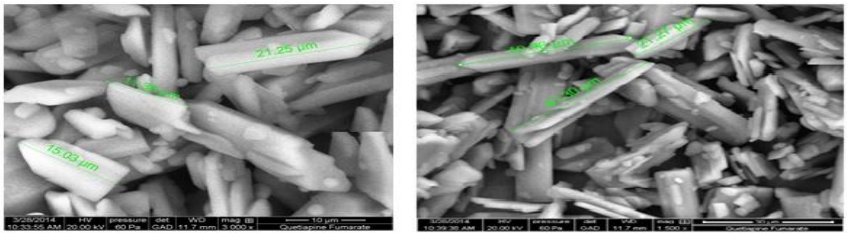


Figure 5: SEM images of Quetiapine fumarate at higher (A) and lower (B) magnification.

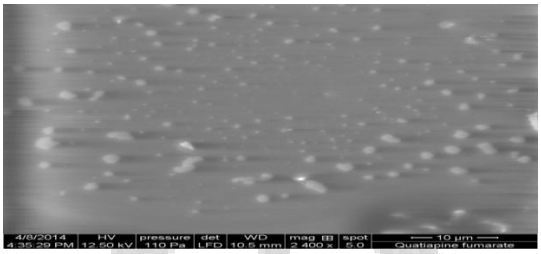


Figure 6: SEM images of Quetiapine fumarate nanosuspension

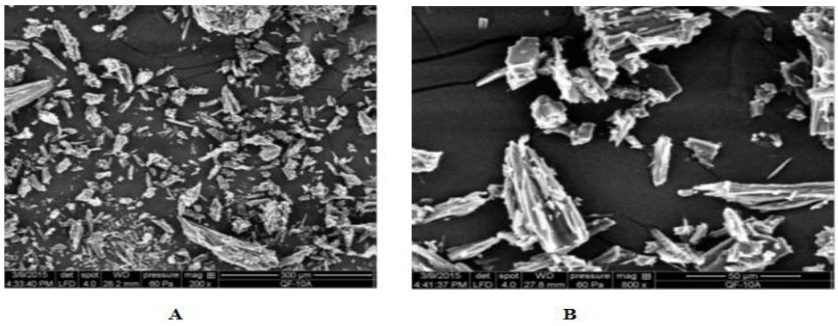


Figure 7: SEM images of QF-010A at lower (A) and higher (B) magnification.

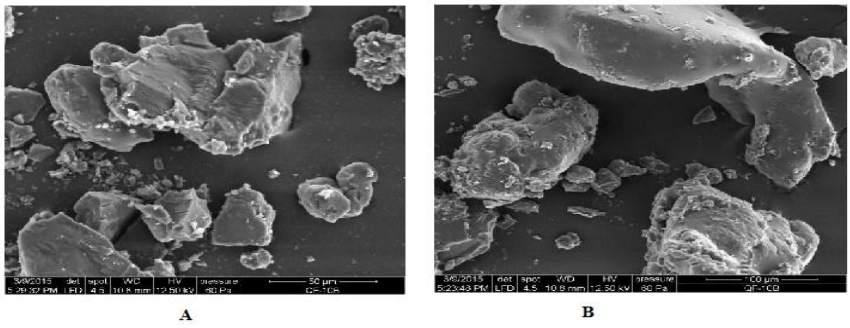
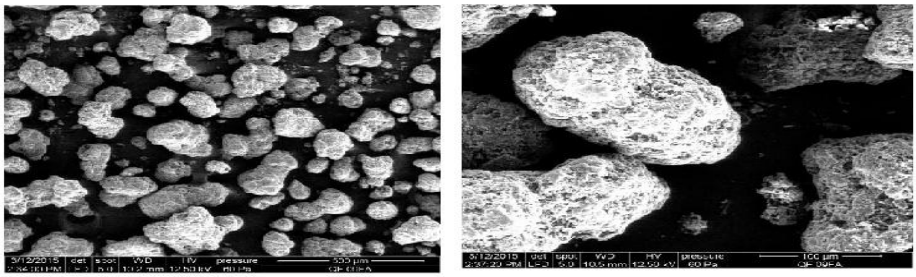
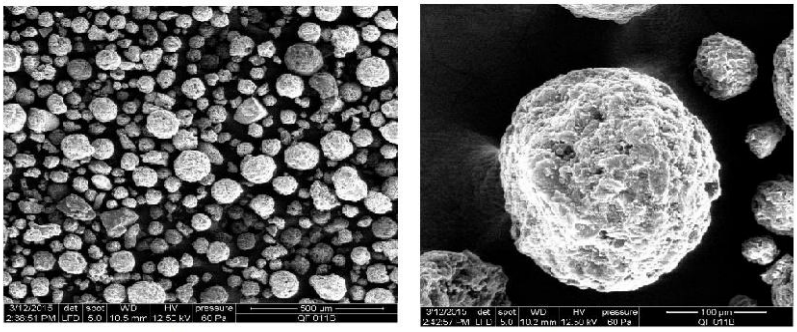


Figure 8: SEM images of QF-010B at lower (A) and higher (B) magnification



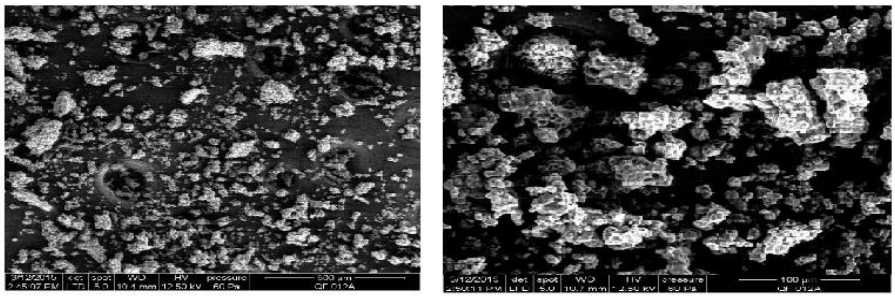
A B

Figure 9: SEM images of QF-011A at lower (A) and higher (B) magnification.



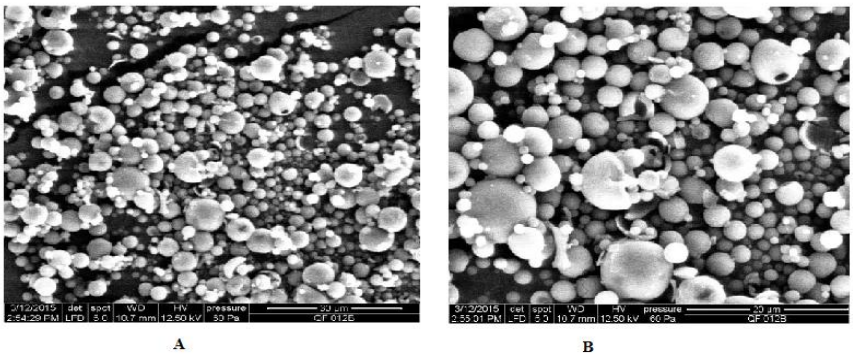
A B

Figure 10: SEM images of QF-011B at lower (A) and higher (B) magnification.



A B

Figure 11: SEM images of QF-012A at lower (A) and higher (B) magnification.



A B

Figure 12: SEM images of QF-012B at lower (A) and higher (B) magnification.

The SEM images of the nanoparticles prepared by spray granulation were shown in figure 9 and 10 using mannitol and lactose as bulking agents respectively. Spray granulated nanoparticles prepared by mannitol show random shaped particles with rounded edges which appeared to aggregate or granules and which had a comparatively larger size ranging from microns to sub-microns particle size. And the spray granulated nanoparticles prepared by lactose as bulking agent shown spherical particles (which appeared to be aggregates or granules) along with some rectangular shaped individual particles. Large size range was observed for these particles. These agglomerates or particle assemblies or granules were composed of a large number of individual nanoparticles.The SEM images of the nanoparticles prepared by spray drying were shown in figure 11 and 12 using mannitol and lactose as bulking agents respectively. Spray dried nanoparticles prepared by mannitol show loose aggregation of particles and random shapes of the particles. The spray dried nanoparticles prepared using lactose as bulking agent show Spherical individual particles are observed which had a smooth outer surface wherein smaller particles were also stacked on larger spherical particles. Size range for these globules varied from micron to low tens of microns (diameter).

Flow properties, moisture and drug content and of dried nanoparticles

Flow properties of dried nanoparticles prepared by three processes with two redispersants i.e. mannitol and lactose; their moisture content and drug content has been determined. Table 2 shows the flow properties, moisture and drug content of the dried nanoparticles compared with the drug.Drug content was also estimated after storing the dried nanoparticles at RT for about 3M. From the data it has been observed the drug quetiapine as such exhibits very poor flow properties and dried nanoparticles have shown significant improvement in the flow properties when compared to the drug as such. Further, it has been observed that the SG/SD nanoparticles have shown better flow properties when compared to the lyophilized nanoparticles. By comparing the drug content of nanosuspension, dried nanoparticles (initial) and dried nanoparticles after storing at RT for about 3M, it has been observed that there is no significant change in the drug content even after drying and storing for about 3M. This has shown that there is no significant degradation of the drug after drying by all the three drying processes.

Table 2: Flow properties, Moisture content and Drug content of dried nanoparticles.

Parameter	Angle of repose (°)	Bulk density (g/ml)	Tapped density (g/ml)	Carr's Index	Hausner Ratio	Moisture content (%)	Drug content (%)		
							NS*	NP #	NP-3M $
Quetiapine Fumarate	-	0.44	0.71	38	1.61	0.98	99.6	99.5	98.2
QF-010A	21.64	0.55	0.735	24.44	1.323	2.14	99.1	98.7	98.1
QF-010B	22.87	0.5	0.675	26	1.351	2.89	99.0	97.4	97
QF-011A	25.6	0.49	0.581	15.68	1.186	1.45	98.9	98.9	98.2
QF-011B	25.16	0.49	0.568	13.72	1.15	1.78	99.2	98.7	98
QF-012A	25.94	0.51	0.568	10.20	1.11	1.08	99.5	99.1	98.3
QF-012B	26.84	0.53	0.609	12.76	1.14	1.07	99.3	98.9	98.1

* NS- Nanosuspension at initial conditions

NP- Dried nanoparticles at initial conditions.

$ NP-3M- Dried nanoparticles after storing at RT for about 3M.

Saturation Solubility:

The saturation solubility of the dried nanoparticles of the drug quetiapine fumarate prepared by different processes such as lyophilization, spray granulation and spray drying was evaluated in 0.1 N HCl, acetate buffer pH 4.5, phosphate buffer pH 6.8, purified water at physiological temperature (37 ^{0}C) and compared with the raw drug. The results from these studies indicated a decrease in drug solubility with increasing pH showing the pH dependent solubility. The higher solubility in acidic pH (0.1N HCl) condition as compared to phosphate buffer pH 6.8 or water could be attributed to the weakly basic nature of Quetiapine fumarate. The saturation solubility of drug nanoparticles was significantly higher than jet-milled microparticles at all pH conditions.The saturation solubility of drug nanoparticles in water is more than double that of the pure drug. Figure 13 shows the saturation solubility of the dried nanoparticles processed by three different processes.

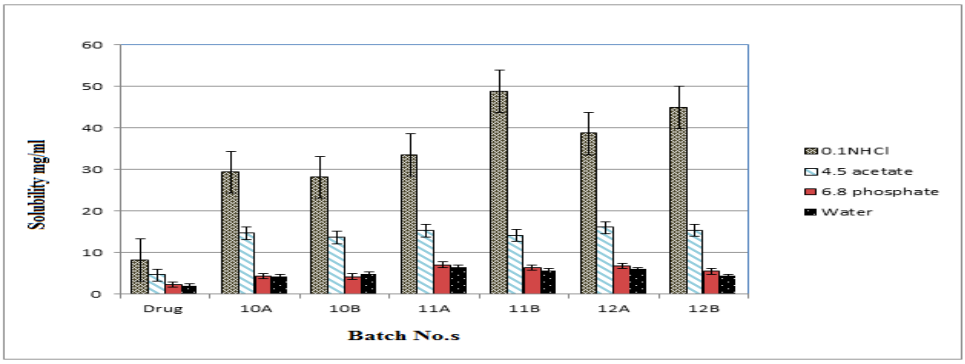


Figure 13: Saturation solubility of dried nanoparticles in different pH media. media. Each number represents mean ± standard deviation, n=3). Standard deviation 1 to 5 %.

In-vitro dissolution

The profiles shown in figure 14 illustrated the dissolution rates of drug quetiapine fumarate and the dried nanoparticles obtained by three different processes such as lyophilization, spray granulation and spray drying. It has been observed that the nanosized drug particles shown increase in the rate and extent of dissolution in comparison with the pure drug especially in initial stage of dissolution i.e within 10 minutes; It has found that only 30% of drug quetiapine fumarate was dissolved whereas quetiapine nanoparticles dried by SG/SD shown more than 70 % dissolved within 10 minutes and more than 40 % of drug dissolved within 10 minutes when dried by lyophilization.

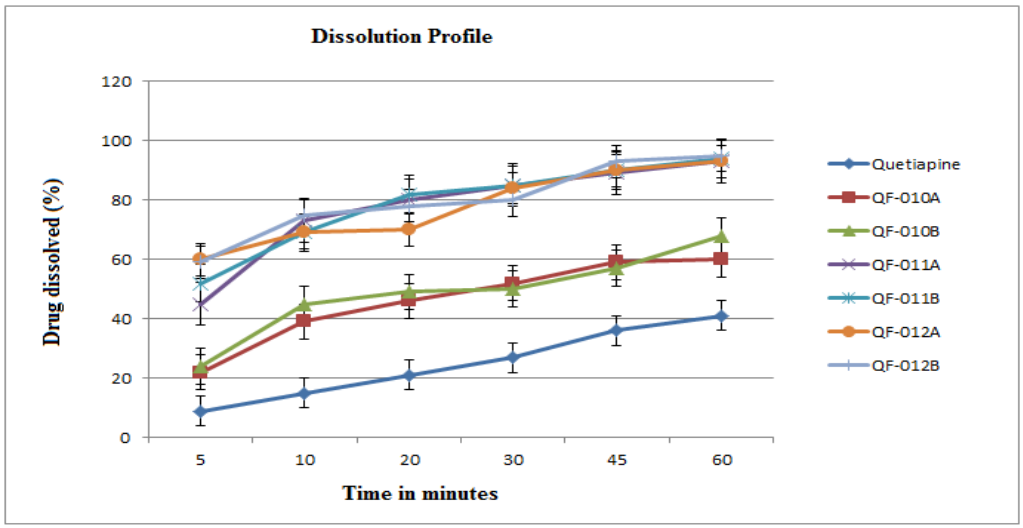


Figure 14: *In-vitro* release profiles of drug and its dried nanoparticles. Each number represents mean ± standard deviation, n=3). Standard deviation 1 to 5 %.

DISCUSSION

The major challenge with the nanosuspension is preservation of physical and the chemical stability of the nanoparticles in the aqueous medium even after converting into the dried nanoparticles. It has been observed that the nanoparticles are prone to physical instability (crystal growth and formation of aggregates) and chemical instability (drug degradation). It has been reported in the literature that on storage of nanosuspension at room temperature, Ostwald ripening occurred. As the nanosuspensions were afflicted by Ostwald ripening and settling, drying of nanosuspension is adopted as a strategy to circumvent these stability issues [xxv]

The nanosuspension was produced by high pressure homogenization using PVP and SLS as surface stabilizers. A combination of PVP and SLS (5:1) was the most successful of all the stabilizing agents investigated as far as the formation of quetiapine suspensions were concerned. From the IR data it has been observed that all the three processes did not change positions of the characteristic bands of the drug, hence the drug remains unchanged after drying also. From previous studies (not captured here) different concentrations and process parameters has been evaluated and based on design of experiments, the concentration of 5 % PVP and 0.75 % SLS and process parameters such as 750barr pressure and 90 minutes of time were found as optimum concentration and process parameters producing smaller stable nanoparticles with narrow particle size distribution. The nanosuspension thus prepared was dried by three different processes i.e lyophilization, spray granulation, spray drying and studied for different parameters.

The mean particle size of the nanosuspension obtained was observed to be 165 nm. From the particle size data it has been observed that after lyophilization, the mean particle size (D50) of the nanoparticles has been increased from 165 nm to 357 nm, this could be attributed to formation of aggregates during lyophilization technique. On storage of the lyophilized nanoparticles at room temperature for about 3M the mean particle size has still increased to 465nm. Whereas the mean particle size of the dried nanoparticles obtained by spray drying or spray granulation was retained at initial conditions and after storing at RT for about 3M, when compared to that of nanosuspension. Further, it was observed that the process of lyophilization when used as drying method broad particle size distribution was obtained this is shown with the high polydispersity index but with both spray granulation and spray drying narrow particle size

distribution was obtained and retained the same after 3M storage. The dried nanoparticles obtained by spray drying or spray granulation after 3M storage retained the narrow particle distribution but the nanosuspension after 3M storage the narrow particle distribution has been changed to broad particle size distribution which is shown by increase of PDI from 0.2 to 0.6. From this data, it has been observed that the nanoparticles after drying only are more stable when compared to nanosuspension. Zeta potential of the nanosuspension was slightly increased after drying the nanosuspension. Zeta potential of the nanoparticles in all the processes observed to be more than 25 owing to stability of the nanoparticles formed. The same trend was observed on stability also. These results suggested that spray granulation and spray drying processes offered better and more promising method of producing quetiapine nanoparticles.

From the XRD and DSC results it has been observed that the all the three processes made the drug loose its crystallinity which was shown by absence of the characteristics peaks and endotherm of the quetiapine fumarate whereas the physical mixture retains the crystallinity. The extra peaks observed in the formulation are matching with that of redispersants (mannitol/lactose) added to the nanosuspension before drying. This is further supported by the SEM analysis wherein the pure drug was shown to have needle shaped crystalline particles, the lyophilized particles still show the needle shaped particles but it may be attributed to that of the redispersants used. Both spray granulated and spray dried processes formed the spherical particles. Further, when the lactose was used as redispersants uniform spherical particles are formed.

From the flow properties of the dried nanoparticles it has been observed that both spray dried or spray granulation processes obtained free flowing powder due to formation of spherical particles, whereas the lyophilization powder yielded poor flowing powder which may be attributed to formation of aggregates or large chunk of particles after lyophilization. From the comparative drug content of the nanosuspension and dried nanoparticles, it has been concluded that there is no significant degradation of the drug after drying by any of the three drying processes. The saturation solubility and *in-vitro* drug release studies have shown that all three processes resulted in increase the solubility or drug release when compared to the pure drug which can be attributed to amorphization and increase in the particle surface area obtained by reducing the particle size. The increase in solubility or drug release was high for spray dried or spray granulated when

compared to lyophilization and was high when lactose used as re-dispersant when compared to mannitol. Spray drying or spray granulation may further be taken up at large scale to evaluate its feasibility in producing stable nanoparticles.

CONCLUSIONS

From the above characterization studies, it has been observed that both spray dried and spray granulation processes are viable approaches to produce stable dried nanoparticles when compared to the lyophilization process. The solid state characterization data showed the absence of drug related peaks or endotherms owing to lose of drug crystallinity or alternatively the drug may be converted to amorphous form. From the drug content data, it has been observed that there is no significant degradation of the drug after drying by any of the three processes. From SEM analyses of spray dried or spray granulated nanoparticles it has been observed that the redispersants used have been precipitated around the drug nanoparticles during drying processes and thus giving the spherical shaped particles thus improving the flow properties of the dried nanoparticles. Further, there was significant enhancement of saturation solubility, *in-vitro* drug release obtained by spray drying or spray granulation when compared to that of pure drug/lyophilized nanoparticles. Among the re-dispersants evaluated lactose has shown more improvement in solubility and dissolution rate, hence it is preferred when compared to mannitol.Out of the three processes evaluated for converting the nanosuspension into dried nanoparticles, spray granulation and spray drying processes are promising method to produce the stable nanoparticles with markedly enhanced saturation solubility and dissolution rate that is attributed to a combination of amorphization and nanonization with increased surface area.

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Declaration of interest

The authors report no declarations of interest.

REFERENCES

1. Zhang W, Yan E, Huang Z, Wang C, Xin Y, Zhao Q, et al. Preparation and study of PPV/ PVA nanofibers via electrospinning PPV precursor alcohol solution", European polymer 2007;43:892-7.
2. Rajesh Singh Tomar, Prateek Chittodiya, Dr. Shikha Agrawal, Pankaj Bahrani;
Solubility Enhancement by Solid Dispersion - A Review International Journal of Pharmaceutical & Biological Archives 2013; 4(4): 623 – 631.
3. Swati Sareen, George Mathew, and Lincy Joseph; Improvement in solubility of poor water-soluble drugs by solid dispersion, Int J Pharm Investig. 2012 Jan-Mar; 2(1): 12–17.
4. G.G. Liversidge, P. Conzentino; Drug particle size reduction for decreasing gastric irritancy and enhancing absorption of naproxen in rats; Int J Pharm, 125 (1995), pp. 309–313.
5. D.R. Paul[a, 1], L.M. Robeson; Polymer nanotechnology: Nanocomposites; Polymer 49 (2008) 3187–3204.
6. Chaubal MV[1], Popescu C. Conversion of nanosuspensions into dry powders by spray drying: a case study, Pharm Res. 2008 Oct; 25(10):2302-8.
7. Keck, C.M., Müller, R.H., 2006. Drug nanocrystals of poorly soluble drugs produced by high pressure homogenization. Eur. J. Pharm. Biopharm. 62, 3–16.
8. Pawar, V. K., Y. Singh, J. G. Meher, S. Gupta, and M. K.Chourasia. 2014. Engineered nanocrystal technology: In-vivo fate, targeting and applications in drug delivery.J. Control. Release 183:51—66.
9. Goren JL, Levin GM. Quetiapine, an atypical antipsychotic. Pharmacotherapy 1998; 18 (6):1183-94.
10. Vishal R. Patel and Y. K. Agrawal; Nanosuspension: An approach to enhance solubility of drugs; J Adv Pharm Technol Res. 2011 Apr-Jun; 2(2): 81–87.
11. Van Eerdenbrugh, B., Vermant, J., Martens, J.A., Froyen, L., Van Humbeeck, J., Augustijns, P., Van den Mooter, G., 2009a. A Screening Study of Surface Stabilization during the Production of Drug Nanocrystals.J.Pharm.Sci., 98, 6, 2091-2103.
12. Wassim Abdelwahed a, GhaniaDegobert a, Serge Stainmesse b, Hatem Fessi Freeze-drying of nanoparticles: Formulation, process and storage considerations; Advanced Drug Delivery Reviews 58 (2006) 1688–1713.
13. Van Eerdenbrugh B, Froyen L, Van Humbeeck J, Martens JA, Augustijns P, Van Den Mooter G. Drying of crystalline drug nanosuspensions-the importance of surface hydrophobicity on dissolution behavior upon redispersion. Eur J Pharm Sci. 2008a;35:127–35.
14. Van Eerdenbrugh B, Van Den Mooter G, Augustijns P. Top-down production of drug nanocrystals: Nanosuspension stabilization, miniaturization and transformation into solid products. Int J Pharm. 2008b; 364:64–75.
15. Van Eerdenbrugh, B.; Vercruysse, S.; Martens, J. A.; Vermant, J.; Froyen, L.; Van Humbeeck, J.; Van den Mooter, G.; Augustijns, P. (2008c). Microcrystalline cellulose, a useful alternative for sucrose as a matrix former during freeze-drying of drug nanosuspensions- A case study with itaconazole.Eur. J. Pharm. Biopharm., 70, 590-596.
16. Chaubal MV[1], Popescu C. Conversion of nanosuspensions into dry powders by spray drying: a case study; Pharm Res. 2008 Oct;25(10):2302-8. Epub 2008 May 29.
17. WANG Wei , CHEN Mo and CHEN Guohua; Issues in Freeze Drying of Aqueous Solutions; Chinese Journal of Chemical Engineering, 20(3) 551—559 (2012)
18. Nekkanti V., Pillai R., Venkateshwarlu V., Harisudhan T. Development and characterization of solid oral dosage form incorporating candesartan nanoparticles. Pharm. Dev. Technol. 2009;14(3):290–298.
19. Carlos E. Figueroa a, Sonali Bose b, Spray granulation: Importance of process parameters on *in vitro* and *in vivo* behavior of dried nanosuspensions; European Journal of Pharmaceutics and Biopharmaceutics 85 (2013) 1046–1055.
20. Kumar AN, Deecaraman M, Rani C. Nanosuspension technology and its applications in drug delivery. Asian J Pharma.2009;3: 168–73.

21. Chen Y, Liu J, Yang X, Zhao X, Xu H. Oleanolic acid nanosuspensions: Preparation, *in-vitro* characterization and enhanced hepatoprotective effect. J Pharm Pharmacol . 2005;57:259–64.
22. Muller et al (2001), Nanosuspensions as particulate drug formulations in therapy. Rational for development and what we can expect for the future, Adv. Drug Deliv.Rev. 47 (1) 3-19.
23. α-Monohydrate Phase in Lactose by DSC **by** Harry G. Brittain, Ph.D and Roger L. Blaine, Ph.D
24. Yuan Listiohadi, James Arthur Hourigan**,** Robert Walter Sleigh, Robert John Steele Thermal analysis of amorphous lactose and α-lactose monohydrate, Dairy Science & TechnologyJanuary 2009, Volume 89, Issue 1, pp 43-67