An Elsevier Indexed Journal ISSN-2230-7346

Journal of Global Trends in Pharmaceutical Sciences

FORMULATION AND *IN-VITRO* CHARACTERIZATION OF RISPERIDONE NANOSUSPENSIONS FOR THE ENHANCEMENT OF DRUG RELEASE RATE

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ARTICLE INFO

Key Words

Dissolution, Nanosuspension, Risperidone, Solubility

ABSTRACT

Poorly water-soluble compounds are difficult to develop as drug products using conventional formulation techniques and are frequently abandoned early in discovery. In the present study, nanosuspensions of risperidone were prepared by nanoprecipitation method for the improvement of solubility and dissolution rate. Through this process the particle size of risperidone can be obtained in the nano-size range, by adjusting the operation parameters, such as the stabilizer concentration. The optimized formulation of nanosuspension obtained is with a stabilizer PVA. The dissolution of nanosized risperidone is significantly enhanced compare with the pure drug. The method was evaluated in comparison with the well-known solvent diffusion process for the efficiency. Control of the preparation variables (stabilizers, drug content, homogenization procedure and cooling conditions) allowed formation of nanosuspensions with diameters less than 300 nm. The major advantage of the nanoprecipitation method over the conventional methods is the avoidance of organic solvents during production, although the mean particle size is slightly greater. The formulations of PVA and Tween 80 as stabilizers yielded nanosuspensions with the smallest average particle size. The formulation of risperidone as a nanosuspension, either in the form of lyophilized powder or granules, was very successful in enhancing dissolution rate, more than 95% of the drug being dissolved in the 60 min compared to less than 15% of the micronized drug. The increase in *in-vitro* dissolution rate may favourably affect bioavailability and improve safety for the patient by decreasing gastric irritancy.

INTRODUCTION:

The design and formulation of a dosage form require consideration of the physical, chemical, and biological characteristics of all the drug substances and pharmaceutical ingredients to be used in its preparation. An important property of a drug substance is solubility, especially aqueous system solubility [1]. One of the critical problems associated with poorly soluble drugs is too low bioavailability and erratic absorption because of their low dissolution rates [2]. The solubility dissolution behavior of a drug is a key factor to its oral bioavailability.

Nanotechnology can be used to solve the problems associated with these conventional approaches for solubility dissolution and bioavailability enhancement. In Nanosuspension technology, the drug is maintained in the required crystalline state with reduced particle size (i.e. increase in the surface area) leading to an increased dissolution rate and therefore improved bioavailability [3]. Reduction of drug particles to nanometer range leads to an enhanced dissolution rate not only because of increased surface area but also because of saturation solubility.

Materials and Methods

Risperidone was obtained as gift from Panacea biotech Ltd. Chandigarh, India. Polyvinyl alcohol, Polyvinyl Pyrrolodine K44, Hydroxymethyl propyl cellulose were procured from Drugs India (Hyderabad, India). All other materials, reagents and solvents used were of analytical grade.

Formulation optimization

The optimized formulation was selected on the basis mean particle size, particle dispersity index and zeta potential and stability.

Preliminary experiments for nanosuspension formulation

Preliminary parameters were optimized by varying one parameter at a time, while keeping other constant, so that effect of various parameters could be evaluated. For optimization of NS, various parameters affecting the nanosuspension like concentration of drug, concentration of zirconium beads, concentration of excipients (different stabilizers were used) and stirring time were studied. The parameters were optimized to obtain nano-ranged size with narrow size distribution [4]. Polymeric stabilizers such as PVA, Tween 80, PVP, and HPMC were taken for study. In order to select the appropriate stabilizer, preliminary experiments with 0.75 %, 1.5 % and 2.0 % (w/v) of various stabilizers were performed. Also, effect of combination of polymeric and surfactant stabilizer (SLS) on mean particle size and zeta potential were investigated. Effect of different concentrations of drug (0.75 %, 1.5 % and 2.0 % w/v) on particle size was investigated. Nanosuspension formulations were prepared by micro precipitation method. The drug is dissolved in suitable organic solvent acetonitrile in which the drug is soluble. This was poured into different amount of water containing different amount of stabilizers and SLS at maintained at room temperature and subsequently stirred on homogenizer to allow volatile solvents to evaporate. Addition of organic solvents by means of a syringe positioned with the needle directly into stabilizer containing water. Organic solvents were left to evaporate off under continuous stirring of the nanosuspension at room temperature for 5 hours [5].

Evaluation of Nanosuspension:

Drug Content: Accurately weighed amount of each preparation dissolved in required amount of methanol and diluted suitably in pH 6.8 phosphate buffer. The drug content was determined Spectrophotometrically at required wavelength.Calculation was done using following formula [6]

$$\% \text{ Drug content} = \frac{\text{Obtained Amount of Drug}}{\text{Theoratical Amount of Drug}}$$

Determination of Nanosuspension Percentage Yield

The nanosuspension production yield was calculated by gravimetric method. Fixed volumes of nanoparticles suspension were centrifuged (15,000×g, 30 min, 15ºC) and sediments were dried [7]. The percentage yield was calculated as follows:

$$\% \text{ Percentage Yield} = \frac{\text{Nano suspension Weight}}{\text{Total Solids Weight}}$$

Particle size measurement

The particle size analysis and particle dispersity index of nanosuspensions were determined using a Malvern Zeta Sizer Nano ZS (Malvern Instruments, Malvern, UK). The particle size index indicates the width of a particle distribution. Prior to the measurement, the samples were diluted with double distilled filtered water to a suitable scattering intensity and re-dispersed by shaking before the measurement. All measurements were performed in triplicate. The results are expressed as mean ± standard deviation (SD).

Analysis of Zeta Potential

The zeta potential is a measure of the electric charge at the surface of the particles indicating the physical stability of colloidal systems. Zeta Potential was measured using a Zeta Sizer Nano ZS (Malvern Instruments, Malvern, UK). Each sample was suitably diluted with double distilled filtered water and placed in a disposable zeta cell. The Zeta potential values were assessed by determining the particle electrophoretic mobility. The electrophoretic mobility was converted to the zeta potential via the Helmholtz Smoluchowski equation. All measurements were performed in triplicate. The results are expressed as mean ± SD.

Solid state evaluation

Changes in the crystalline state can affect the solubility, dissolution velocity, the oral bioavailability as well as the stability of a pharmaceutical formulation. Therefore crystalline structure of nasuspension was investigated by DSC.

Differential Scanning Calorimetry

DSC scans of prepared dried powdered drug sample, pure drug and physical mixture were recorded using DSC Hitachi 7020 with muse software. All samples were weighed (8-10mg) and heated at scanning rate of 10^{o}C/min under dry nitrogen flow (50ml/min). Aluminum pans and lids were used for all samples.

***In - Vitro* Drug Release Studies**

In-vitro dissolution study of nanosuspensions formulations was carried out using USP dissolution apparatus type II. Studies were carried out using phosphate buffer pH 6.8 as dissolution medium (900ml) and 50 rpm was set throughout the study. Samples were withdrawn at regular time interval of 10, 20, 30, 40, 50 and 60 minutes. Samples were replaced by equivalent volume of fresh dissolution medium. The withdrawn samples were spectrophotometrically analyzed at respective wavelength on UV Spectrophotometer [8].

Drug and excipient compatability studies by FTIR

FTIR spectra were performed using Bruker FTIR spectrophotometer to find out any possible drug-stabilizer interactions. Samples of drug and of each stabilizer used as well as samples of the prepared nanosuspensions were grounded separately and mixed thoroughly with potassium bromide. The ratio of sample and KBr was kept for all the preparations. The potassium bromide discs were prepared by compressing the powders at a pressure of 5 tones for 5 min in a hydraulic press. Scans were obtained from 4000 to 500 cm-1.

Scanning Electron Microscopy

Surface morphology of nanosuspensions was investigated using Scanning Electron Microscopy (SEM). The samples for SEM study were prepared by lightly sprinkling the formulation on a double-adhesive tape stuck to an aluminum stub. The stubs were then coated with gold to a thickness of ~300 Å under an argon atmosphere using a gold sputter module in a high vacuum evaporator. The coated samples were then

randomly scanned and photomicrographs were taken with a scanning electron microscope. SEM was operated at an acceleration voltage of 20 kV [9].

RESULTS AND DISCUSSION

The nanosuspension formulations prepared with no stabilizer showed rapid agglomeration of drug particles instantly after preparation. The agglomeration of particles is may be due to the attractive forces between the particles in the absence of significant energy barrier, in addition to above it might also due to so-called hydrophobic effect. The presence of hydrophobic particles or molecules in water causes distortion and rearrangement of hydrogen bonding in the aqueous medium, thus greatly increasing the free energy of the system. As a result, these hydrophobic particles tend to agglomerate to reduce the system free energy.

Four stabilizers (PVA, Tween 80, HPMC and PVP) were tested for their stabilization potential. Important function of stabilizer is that they can form a substantial mechanical and thermodynamic barrier at the interface that retards the approach and coalescence of individual nanoparticles. As per results obtained, it may be concluded that mean particle size varies with stabilizer and with PVA it shows lowest size followed by then the other stabilizer. As shown in results an appropriate amount of stabilizer is required to achieve smaller particle size. The crystal growth was protected by the adsorbed stabilizers, and the quantity of stabilizer should be enough to cover the crystal surface to provide enough steric repulsion between the crystals. Inadequate surface coverage of stabilizer could result in rapid crystal growth and agglomeration, while high concentration of stabilizer could result in enhanced viscosity of the solution which would obstruct the diffusion between the solvent and anti-solvent during precipitation. The type of compound and their amount employed for stabilization has a prominent effect on particle size. At the low drug concentration, the particle size was smaller with a narrow size distribution. However, at the higher drug concentration, due to greater supersaturation, a higher diffusion controlled growth and agglomeration rate were achieved, resulting in larger crystals. Stirring speed is obviously affecting the particle size, as increasing the stirring speed, decrease in mean particle size because of high shear force.

Role of surfactant in formulation

In precipitation which is bottoms up technique nanosuspensions are formed by building particles up from molecular stage. During this new surface area is formed, which produces high free-energy, leading to agglomeration of particles and subsequent increase in particle size. This tendency can be avoided by addition of surface active agents, which reduces the free surface energy. Hence, during the formulation of nanosuspension the optimization of surface active agents has prime importance.

Fourier Transform Infrared Spectroscopy (FTIR)

FTIR spectroscopy Figures 1(a)–1(h) revealed that there is neither appearance of new peaks nor disappearance of existing peaks, which indicated that there is no interaction between the drug and each of the stabilizers used. The characteristic ketone (C=O) stretching vibration at 1747.22 cm^{-1}, C-H bending at 1412.57 cm^{-1} , C-O stretching at 1261.59 cm^{-1} , C-N vibration at 1313.29 cm^{-1} , aromatic C-H stretching at 747.69 cm^{-1} and the stretching band in the region of 3500-3200 cm^{-1} assigned to the non bonded aromatic amino group were identified in all the spectrums of the nanosuspensions.

Particle size analysis

The mean particle size varies 248±4.78 nm to 358±4.28 nm and showed good correlation coefficient (0.8935. The particle size of different formulation was shown in table 2, which clearly indicates the batch F2 had less particle size as compare to other formulation.

Table 1: Composition of risperidone nanosuspensions

Batch Code	Stabilizer	Drug (mg)	Drug: Stabilizer
F1	NS without Stabilizer	20	-
F2	PVA	20	1:0.4
F3	Tween 80	20	1:0.4
F4	PVP K44	20	1:0.4
F5	HPMC K4M	20	1:0.4

Table 2: Drug content, percentage yield, particle Size (P.S.), and size distribution of nanosuspensions prepared

Formulation Code	Stabilizer	Drug Content %	% Yield	P.S. in nm	Polydispersibility Index
	Risperidone	-	-	3548±25.15	0.51
F1	NS without Stabilizer	62.38	99.6	1264±64.85	1.00
F2	PVA	96.67	94.3	248±4.78	0.48
F3	Tween 80	94.78	95.7	346±3.48	0.38
F4	PVP K44	88.49	93.1	358±4.28	0.69
F5	HPMC K4M	90.78	95.6	346±3.34	0.58

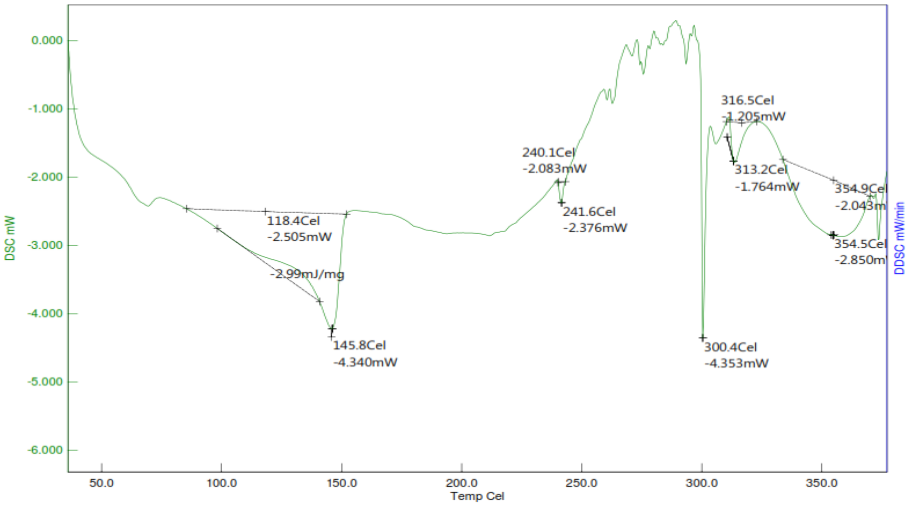

Figure 1: Drug and PVA mixture DSC image

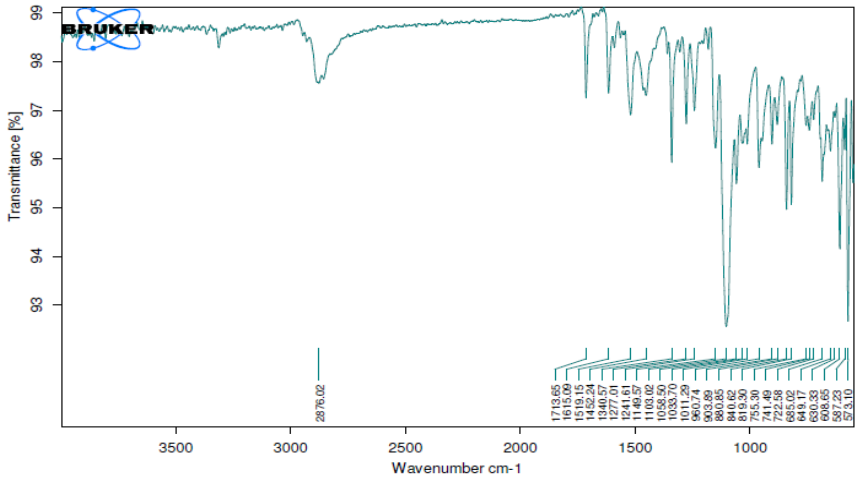

Figure 2: Risperidone Drug+ PVA FTIR

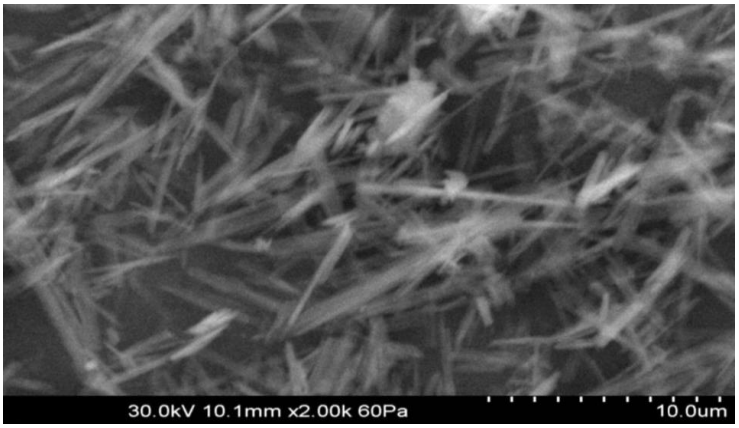

Figure 3: Risperidone Pure Drug

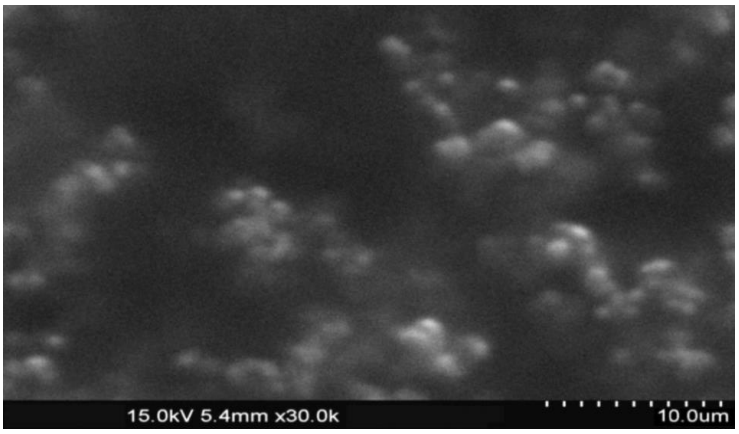

Figure 4: Risperidone nano suspension formulation

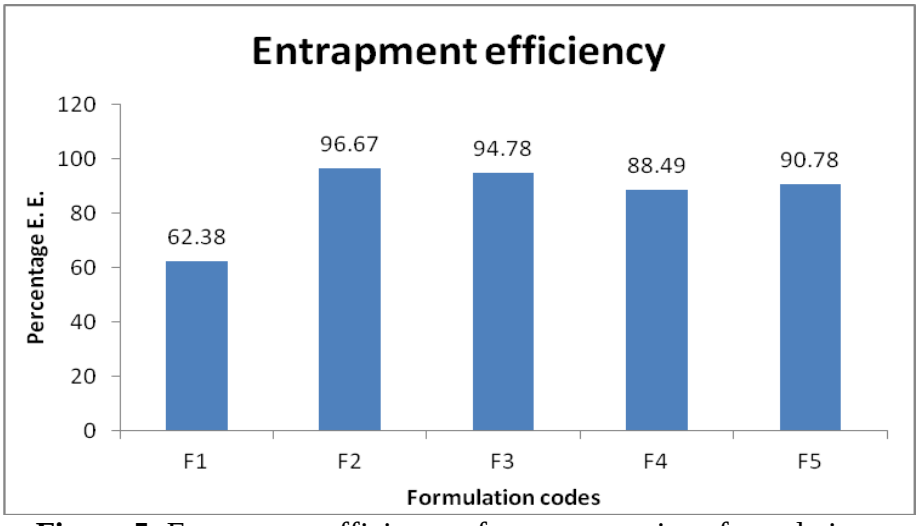

Figure 5: Entrapment efficiency of nanosuspensions formulations

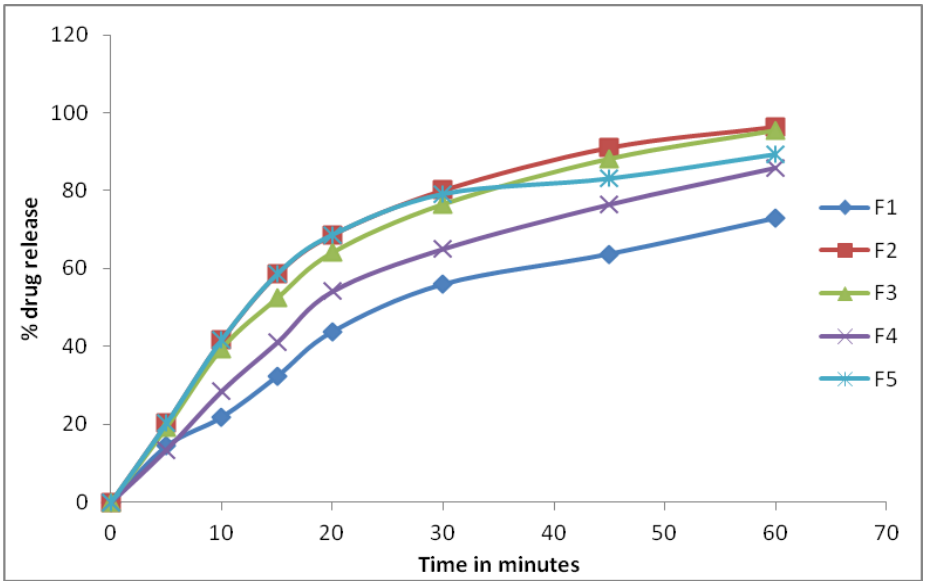

Figure 6: Percentage drug release profile for formulations in phosphate buffer

The batch F3 had a Z-average particle size of 346 nm. Results of the mean particle size equation indicate that the all three independent variable significantly affect the mean particle size. Insufficient surface coverage of stabilizer could result in rapid crystal growth and agglomeration, while high concentration of stabilizer could result in enhanced viscosity of the solution. However, drug concentration was more significantly affect the mean particle size because at the higher drug concentration, due to greater supersaturation, a higher diffusion controlled growth and agglomeration rate were achieved, resulting in larger crystals.

Zeta Potential

It is generally acknolowdged that a zeta potential of approximately ± 20 mV is required. Zeta potential analysis was performed to get information about the surface properties of nanosuspensions. The zeta potential of the prepared nanosuspensions was found to be +21.29 mV. This value indicates the stability of the formulation and it is closer to the required zeta potential value.

Scanning Electron Microscopy

Pure drug and nanosuspensions surface morphology and shape were analyzed by SEM, representative examples are shown in figure 2 and 3. It can be seen that the raw drug particles existed as irregular tabular and prismatic crystals with smooth surface. Micronized pure form of drug powder showed irregular shapes with particle size generally larger than the prepared nanosuspensions and with a broad particle size distribution. The prepared nanosuspensions were more uniform in shape as compared to pure drug but with more or less rough surfaces. The images revealed the presence of aggregates or particle assemblies which were composed of a large number of individual nanoparticles. The weakened steric hindrance effects, derived from the thinner stabilizer layer, would probably result in partial aggregation among nanosuspensions, which would increase the particle size distribution of nanosuspensions.

Drug Content

During the preparation process there was no any drug loss step involved, so theoretically the formulation was considered as being 100% drug content. Table 1, demonstrates the drug content for each nanosuspension formulation prepared. It is obvious that the drug content of nanosuspension can be considered as a function of both the nature of the stabilizer used as well as the method of preparation. According to the data of

table 1, it can be seen that all the drug content were within acceptable limit.

***In-vitro* dissolution studies**

Dissolution studies were compared for pure drug, and optimized nanosuspension formulation. The amount of drug released from the optimized nanosuspension formulation was 96.41% within 60 min compared to amount of 15.82 % of pure drug after 1 hour in phosphate buffer pH 6.8. The increase in accessible surface area to the dissolution medium and hydrophilic surfactant coating on the particle surfaces may be the reason for increase in dissolution rate. This enhanced dissolution rate can be attributed to the higher surface area of nanocrystals available for dissolution and the decreased diffusion layer thickness.

CONCLUSION

Risperidone nanosuspensions were prepared by nanoprecipitation. Nanoprecipitation technique has been described as a simple method for drug nano-sizing at laboratory scale. Preliminary trails helped in identifying the significant parameters that affected the response variables. All the predetermined independent variables except drug concentration were found to affect the dependent variables. Particle size is significantly influenced by concentration of drug, concentration of stabilizer and starring speed. Nanosized risperidone dissolved significantly faster than pure drug powder. The optimized formulation maintained the crystallinity of risperidone and released almost 96.41 % drug within 60 minutes.

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