# Nanoteknologi Farmasi Ziprasidone Nanocrystals By Wet Media Milling Followed By Spray Drying And Lyophilization: Formulation And Process Parameter Optimization

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## ABSTRAK

liofilisasi. Metode penelitian yang digunakan meliputi pembuatan nanosuspensi Ziprasidone menggunakan teknik pengurangan ukuran, pengoptimalan parameter proses, formulasi teknik penggilingan media, serta evaluasi stabilitas fisik nanosuspensi yang telah dioptimalkan. Hasil dari penelitian ini diharapkan dapat memberikan pemahaman yang lebih baik tentang penggunaan nanokristal dalam meningkatkan kelarutan dan bioavailabilitas obat dengan kelarutan yang buruk seperti Ziprasidone. Selain itu, penelitian ini juga diharapkan dapat memberikan wawasan baru dalam penggunaan teknologi pengeringan semprot dan liofilisasi untuk meningkatkan stabilitas fisik nanosuspensi obat. Kesimpulannya, penelitian ini menyoroti pentingnya penggunaan nanokristal dalam meningkatkan kelarutan dan bioavailabilitas obat dengan kelarutan yang buruk seperti Ziprasidone. Metode pengeringan semprot dan liofilisasi telah terbukti efektif dalam meningkatkan stabilitas fisik nanosuspensi obat. Penelitian ini dapat menjadi landasan bagi pengembangan formulasi obat yang lebih efektif dan dapat digunakan dalam aplikasi klinis yang lebih luas.

**Kata Kunci** : Nanoteknologi Farmasi, Liofilisasi

## ABSTRACT

lyophilisation. The research methods used include the preparation of Ziprasidone nanosuspension using size reduction technique, optimisation of process parameters, formulation of media milling technique, and evaluation of the physical stability of the optimised nanosuspension. The results of this study are expected to provide a better understanding of the use of nanocrystals in improving the solubility and bioavailability of drugs with poor solubility such as Ziprasidone. In addition, this study is also expected to provide new insights into the use of spray drying and lyophilisation technologies to improve the physical stability of drug nanosuspensions. In conclusion, this study highlights the importance of using nanocrystals in improving the solubility and bioavailability of drugs with poor solubility such as Ziprasidone. Spray drying and lyophilisation methods have been shown to be effective in improving the physical stability of drug nanosuspensions. This research can serve as a foundation for the development of more effective drug formulations that can be used in wider clinical applications.

**Keyword** : Pharmaceutical Nanotechnology, Lyophilization

## INTRODUCTION

The most important issue in drug discovery and development is poor solubility. Many new chemical entities show significant therapeutic effects, better efficiency but their clinical applications are limited due to poor solubility of active ingredients (Peltonen et al., 2018). Supported by the study of Shinde et al. (2019) solubility, dissolution and gastrointestinal permeability are fundamental parameters that control the rate and extent of drug absorption and its bioavailability. Poor water solubility is a major challenge for formulation development, hence alternatives are required to improve the oral bioavailability of poorly soluble drugs.

Ziprasidone is an atypical antipsychotic agent used for the treatment of schizophrenia, mania and bipolar disorder. Ziprasidone has high safety and efficacy, but low oral bioavailability (60%). Ziprasidone has high lipophilicity (c log = 3.6) and poor solubility (intrinsic solubility 0.3 μg/ml) its solubility in simulated biorelevant liquids is estimated to be 4-5 μg/ml. Thus Ziprasidone exhibits limited solubility and absorption dissolution rate (Koradia et al., 2018).

One attempt to overcome the solubility of Ziprasidone and improve its dissolution is to form nanocrystal preparations. Nanocrystals as a formulation option for drugs that exhibit poor solubility and dissolution rate. Nanocrystals consist of submicron-sized crystalline drug particles stabilized in a liquid medium, usually water. They can be produced either by precipitation techniques (bottom-up approach) or by size reduction (top-down approach). Drug nanocrystals are nanoparticles consisting of 100% drug without matrix material and the average particle size is below 1μm (Dhole et al., 2019).

Based on the description above, the authors are interested in further discussing Ziprasidone nanocrystals. This aims to improve the poor aqueous solubility of Ziprasidone as well as its bioavailability, through size reduction to the nano range. Therefore, this paper will describe the formulation and optimization of process parameters by spray drying and lyophilization.

This study aims to achieve several objectives which include understanding the method of improving the solubility of Ziprasidone nanosuspension, optimizing the process parameters and formulation of media milling technique, and improving the physical stability of the optimized nanosuspension using spray drying and lyophilization method.

## METODE

### Preparation of Nanosuspension by Media Milling Method

Nanosuspensions were prepared by wet media milling technique. The drug (2% b/v) was dispersed in an aqueous solution containing 1% b/v stabilizer. The resulting suspension was poured into a glass bottle containing zirconium oxide beads as milling media and stirred at a steady speed using a magnetic stirrer for a predetermined period of time.

### Preliminary Work for Variable Screening

Preliminary studies were conducted to determine the ideal conditions for the preparation of nanosuspensions by wet media milling. The parameters studied were the amount of milling agent, milling time, suspension volume, stirring speed and type of stabilizer.

**Amount of Milling Agent and Milling Time**

Zirconium oxide beads were used as milling agents. Nanosuspensions were prepared by varying the number of milling beads. The milling operation was performed by taking four different amounts of milling beads (2.5, 5, 7 and 10 g m-3) and samples were taken at different time intervals (3, 6, 9 and 12 h) and the particle size and PDI were determined.

**Water Volume**

Nanosuspensions were prepared by taking different volumes of water (5, 7 and 10 ml; batch b17, b18 and b19). Milling was carried out by taking 7 gm of Zirconium oxide granules and a stirring speed of 1000 rpm. Poloxamer 407 (1% b/v) was used as stabilizer and milling was carried out for 6 h for the preparation of ziprasidone nanosuspension and the prepared nanosuspension was evaluated with respect to particle size and PDI.

**Stirring Speed**

Milling operations were carried out at four different milling speeds (500, 1000, 1500 and 2000 rpm; batches b20, b21, b22 and b23) and the prepared nanosuspensions were evaluated with respect to particle size and PDI.

**Type of Stabilizers**

Particle stability and physical properties of particles depend on the stabilizer used. In this study different polymeric stabilizers like PVA, PVP K-30, HPMC, non-ionic surfactants like Poloxamer 407, Poloxamer 188, Vitamin E TPGS and tween 80 and ionic surfactants like SLS were taken for selection of suitable stabilizer. The prepared nanosuspensions were evaluated with respect to particle size and zeta potential.

**Drying of Nanosuspensions**

The optimized batch from the screening study is subjected to solidification by spray drying and lyophilization processes.

**Spray Drying**

Solidification of the nanosuspensions containing 5% b/v mannitol as matrix formers was carried out with a lab-scale spray drier. The process conditions set were inlet temperature: 110˚C, outlet temperature: 60˚C, flow rate: 1 ml/min, aspiration: 50 $^{Nm3/H}$.

## Lyophilization

Lyophilization of nanosuspensions was performed using 5% b/v mannitol as cryoprotectant. The samples were frozen at -45˚C for 12 hours, primary drying was done at -20˚C for 6 hours and secondary drying was done at 20˚C for 6 hours.

## Nanosuspension Characterization

### Particle Size and Size Distribution

Particle size and Polydispersity index (PDI) were measured by *Particle Size Analyzer* (PSA). All samples were diluted with distilled water and then sonicated for about 1 minute to prevent aggregation. All measurements were taken in triplicate and the average then standard deviation was calculated.

### Zeta Potential

The zeta potential of the samples was assessed by measuring the electrophoretic mobility of the particles using a *Particle Size Analyzer* (PSA).

### Drug Excipient Compatibility

Drug excipient compatibility studies were carried out by *Fourier Transform Infrared Spectroscopy* (FTIR) and *Differential Scanning Calorimetry* (DSC) to evaluate the possible interactions between drug and excipients used in the formulation. Studies were conducted for Ziprasidone, Vitamin E TPGS, and a physical mixture of Ziprasidone and Vitamin E TPGS (1:1).

#### *Fourier Transform Infrared Spectroscopy (FTIR)*

FTIR analysis was performed using the potassium bromide (KBr) disk method. In this method the sample was mixed with KBr and this mixture was converted into a disk using a KBr press. The *disk* was kept in the sample holder of the FTIR spectrophotometer and scanned in the range of 400-4000 $^{cm-1}$ with a resolution of 4 $^{cm-1}$.

#### *Differential Scanning Calorimetry (DSC)*

DSC of all the samples obtained 2.5 mg sample was accurately weighed and sealed in a standard aluminum crucibles with a single hole in the lid. An empty pan of the same type was used as a reference. The DSC instrument was calibrated using indium as a standard (melting point = 156.6 ± 0.3°C). DSC scans of each sample were performed at a heating rate of 10-C/min over a temperature range of 45-300°C. The DSC cell was purged with a stream of nitrogen at a rate of 50 mL $^{min-1}$.

## Characterization of Solid Nanosuspensions

### Redispersibility

Solidified nanosuspensions (Spray dried or lyophilized) were dispersed in distilled water and shaken vigorously. The particle size of the dispersed nanoparticles was measured

and the percent redispersibility index (RDI%) was calculated according to the equation RDI% = ×100, where Z0 is the diameter of the nanosuspension before drying and Z is the diameter of the reconstituted nanosuspension (Toziopoulou, F., et al, 2017).

**Saturated Solubility**

Saturated solubility was performed by the shake flask method. Excess amounts of pure ziprasidone, ziprasidone nanosuspension, lyophilized nanosuspension and spray dried nanosuspension were dispersed in phosphate buffer pH 7.5 and shaken at 50 rpm and 37°C for 72 hours using an orbital shaking incubator. The samples were then centrifuged at 5000 rpm for 10 min, the supernatant was filtered and analyzed by UV spectroscopy (Yue, P.F., et al, 2013).

***In Vitro* Dissolution Study**

Performed using USP type II apparatus (paddle) in 900 ml of 0.05 M Sodium Phosphate buffer pH 7.5 at 37±0.5°C and rotation speed of 50 rpm. A sample equivalent to 100 mg of drug was introduced directly into the dissolution medium. At predetermined time intervals (5, 10, 15, 30, 60, 90, 120 and 150 min) 5 ml of sample was withdrawn and replaced with fresh medium. The samples were filtered and analyzed by UV spectrophotometer at a wavelength of 319.8 nm using 0.05 M Sodium Phosphate buffer pH 7.5 as a blank.

**X-ray Diffraction Study**

X-ray diffraction analysis was carried out to detect the crystallinity of pure Ziprasidone, spray dried Ziprasidone nanosuspension and lyophilized Ziprasidone nanosuspension using an X-Ray diffractometer. The powders were placed in a sample holder and scanned between 10 and $^{80o}$ (2q).

***Scanning Electron Microscopy (SEM)***

Morphology of Ziprasidone nanosuspension, spray dried and Lyophilized Ziprasidone nanosuspension was performed by Field Emission Scanning Electron Microscopy. Images were acquired in a legal vacuum model under an accelerating voltage of 30 kV.

**RESULTS AND DISCUSSION**

**Effect of Substance Amount and Milling Time on Particle Size and PDI**

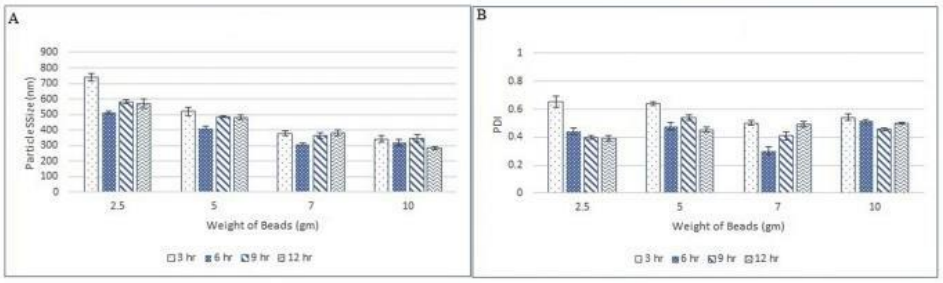

**Figure 1.** Effect of milling agent amount and milling time on particle size and PDI of Ziprasidone nanosuspension

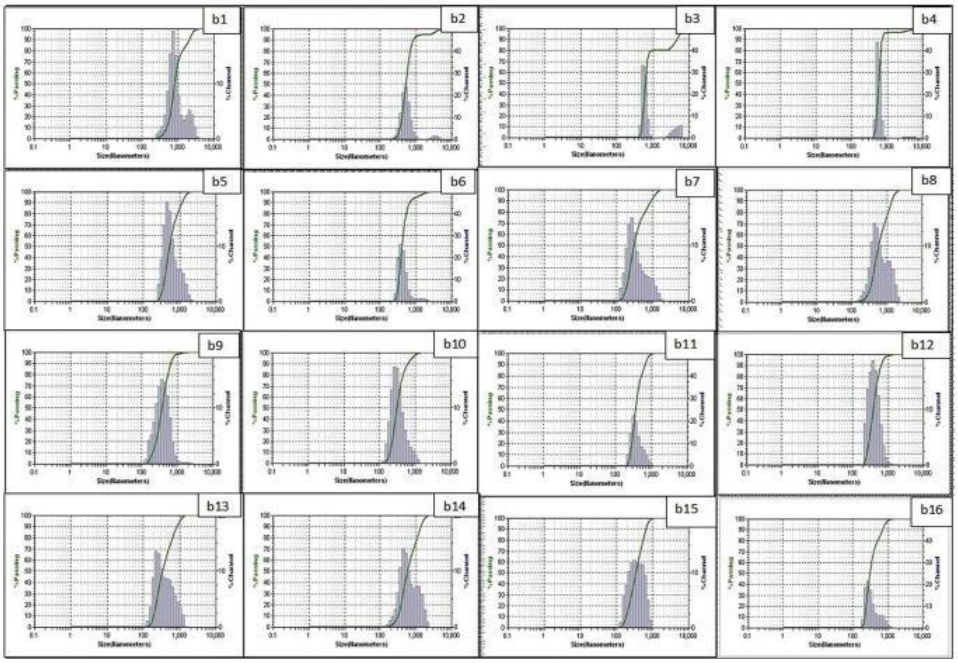


**Figure 2.** Particle size distribution graph of batch b1-b16

Figure 1 and Figure 2 show the effect of milling time and amount of milling material on particle size and PDI for batches b1 to b16. It was observed that a milling time of 6 hours was sufficient to obtain a smaller average particle size, but the longer milling time caused aggregation due to the high kinetic energy of the particles. On increasing the amount of zirconium oxide beads from 2.5 to 7 gm, the particle size and PDI (at 6 h milling time) reduced because as the amount of milling media increased, the contact point between the beads and particles increased which increased collisions. Eventually reducing the particle size and PDI but further increasing the amount of zirconium oxide from 7 to 10 gm the particle size and PDI slightly increased due to the repulsive force between the particles which made the particles unstable and formed aggregates. Based on these results, 7 gm zirconium oxide beads and 6 h milling time were selected for further study.

**Effect of Water Volume on Particle Size and PDI**

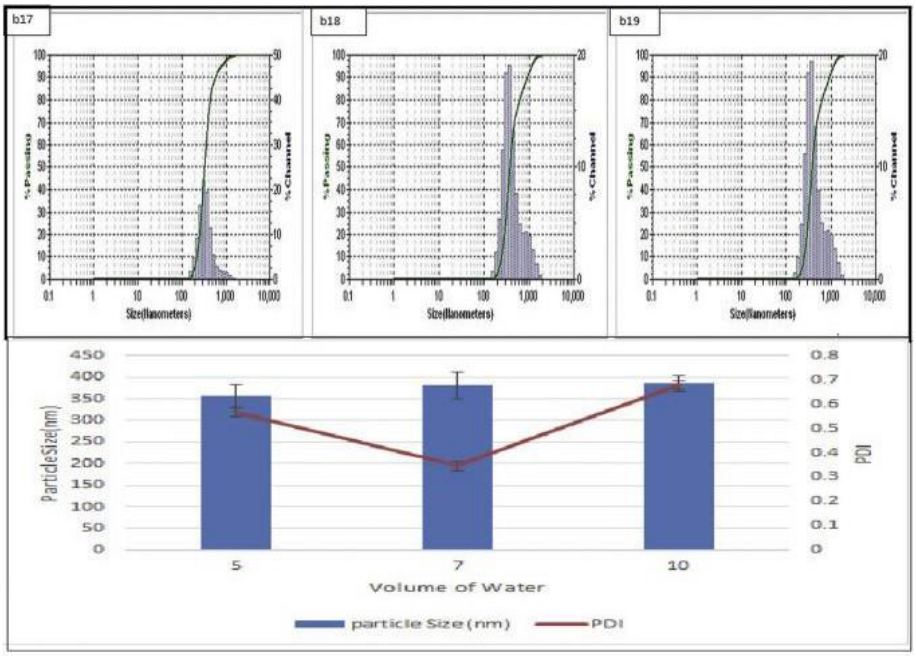


**Figure 3.** Effect of water volume on particle size distribution of Ziprasidone nanosuspension

No significant difference in particle size was observed on increasing water volume only a slight increase in particle size (356 nm, 381 nm, 386 nm for batches b17, b18 and b19 respectively) was observed with increasing water volume which is due to the effect of less solid content in the suspension resulting in less friction between solid particles. Based on the results 5 ml of water volume was selected for further study.

**Effect of Stirring Speed on Particle Size and PDI**

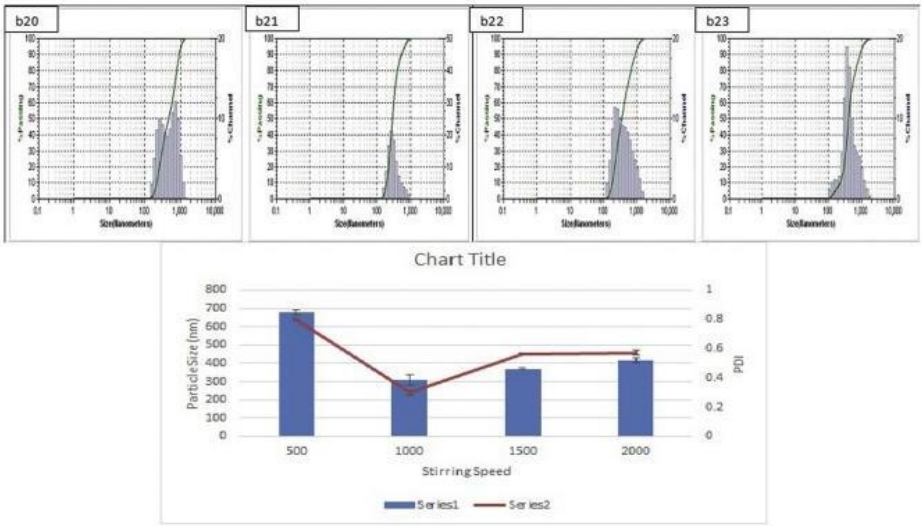


**Figure 4.** Effect of stirring speed on particle size distribution of Ziprasidone nanosuspension

Batch b20, prepared at a stirring speed of 500 rpm showed a particle size of 678 nm while it was 310 nm, 369 nm and 415 nm for batch b21, b22 and b23 respectively which means on increasing the stirring speed from 500 rpm to 1000 rpm the particle size reduces, this is due to greater force on individual drug particles at higher milling rpm than at lower rpm. Thus more energy is available within the grinding chamber to reduce particle size through impaction, abrasion and cleavage at higher grinding rpm than at lower rpm. On increasing the milling speed above 1000 rpm, the particle size increased slightly hence a milling speed of 1000 rpm was selected for further study.

**Stabilizing Agents**

The decrease in particle size to the nanoscale creates a high-energy surface that leads to particle aggregation and Oswald ripening thus stabilizers are required in the production of nanosuspensions. Polymers or surfactants (ionic and nonionic) are commonly used as stabilizers in nanosuspension stabilization. Based on the stabilization mechanism, stabilizers are classified into two main groups, namely steric stabilizers and electrostatic stabilizers. In this study polymeric stabilizers such as PVA, PVP K-30, HPMC; nonionic surfactants such as Poloxamer (407 and 188), Vitamin E TPGS and tween 80 and ionic surfactant SLS were screened. From the particle size analysis (as shown in Figs. 5 and 6) it was observed that the polymer stabilized nanosuspension formulation containing PVP K-30 (batch: b25) showed higher particle size (500 nm) compared to the formulation containing HPMC (batch: b26, 468 nm) and formulation containing PVA (batch: b24, 377 nm). These results are in accordance with several other studies which show that the difference in particle size may be due to the difference in structure, namely PVA and HPMC have a flexible long chain-like structure while PVP K-30 has a roll-like structure so that the surface absorption properties of PVA and HPMC are better than PVP K-30. Among the nonionic surfactants, the minimum particle size distribution was observed with Vitamin E TPGS (252 nm, batch: b29) which is due to its low viscosity and high surface activity.

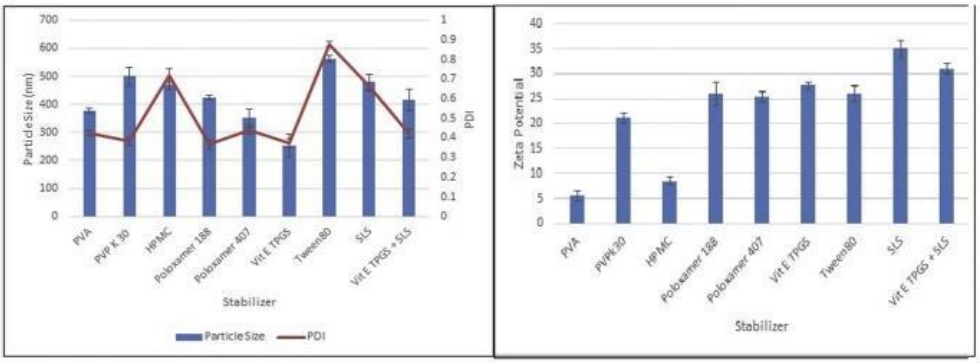


**Figure 5.** Effect of stabilizer type on particle size distribution and Zeta potential and Ziprasidone nanosuspension

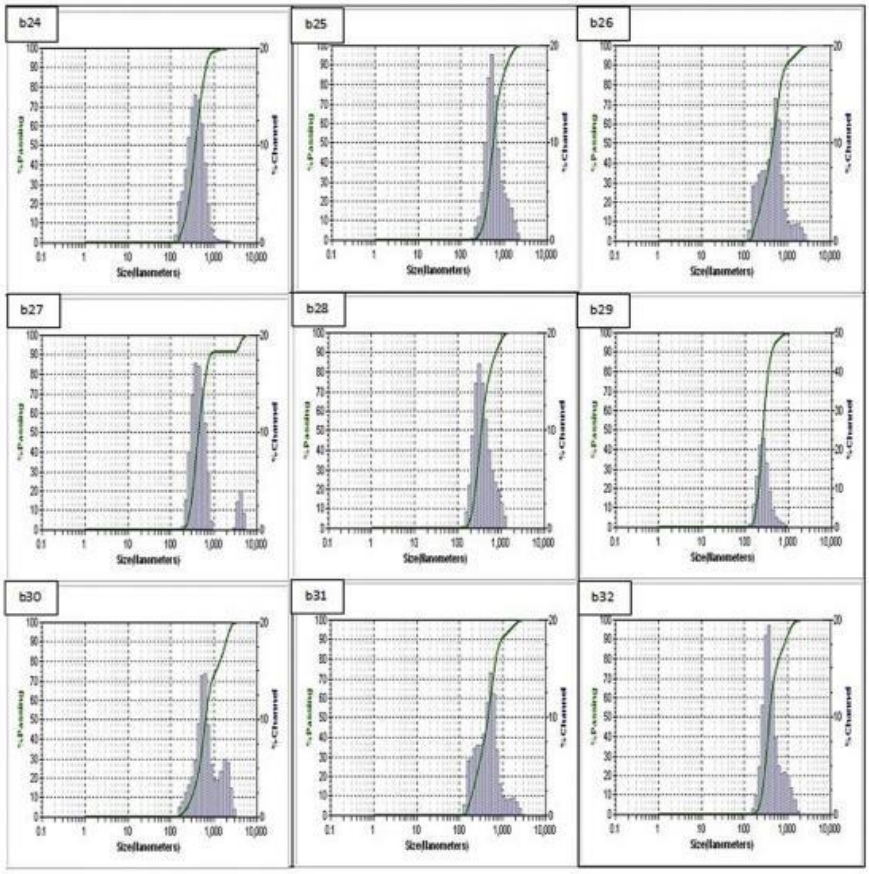


**Figure 6.** Particle size distribution graph of batch b24-b32

The particle size observed in tween 80 (562 nm, batch: b30) is due to the lower molecular weight of tween 80. Among the two Poloxamer grades, Poloxamer 407 showed lower particle size (354 nm, batch: b28) than Poloxamer 188 (425 nm, batch: b27) this is due to the difference in molecular weight, Poloxamer 407 has higher molecular weight and lower hydrophilic lipophilic balance (HLB=18-23) than Poloxamer 188 so the surface coverage of Poloxamer 407 is more compared to Poloxamer 188. The zeta potential of batches b24 to b30 is shown in Figure 5. The zeta potential of the nanosuspension stabilized with Vitamin E TPGS was about 27 mv (Figure 5). The zeta potential is an index of the physical stability of a dispersed system because it calculates the electrostatic resistance that can prevent nanoparticles from aggregation and agglomeration. A Zeta potential of at least ±30 mv for electrostatically stabilized systems or ±20 mv for sterically stabilized systems is sufficient to fully stabilize the nanosuspension system. completely. The zeta potential is reduced in the case of sterically stabilized suspensions due to the absorbed stabilizer layer which shifts the shear plane to a greater distance from the particle surface. The combination of Vitamin E TPGS and SLS showed larger particle size (415 nm, batch: b32) compared to Vitamin E TPGS alone hence Vitamin E TPGS was used for further studies.

***Fourier Transform Infrared Spectroscopy (FTIR)***

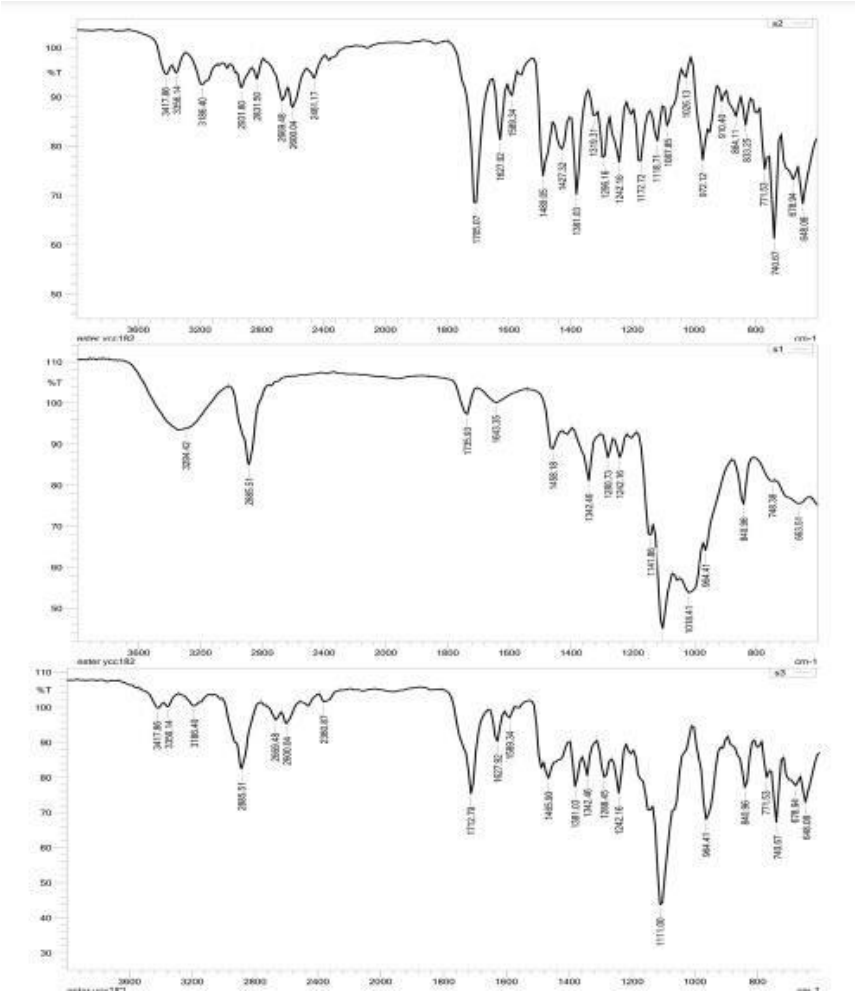

**Figure 7.** FTIR Spectrum and Ziprasidone, Vit. E TPGS, Physical mixture of Ziprasidone and Vitamin E TPGS.

The results showed that all the characteristic peaks of the drug were retained in the physical mixture indicating the compatibility of the drug with Vitamin E TPGS.

## Redispersibilitas

**Table 3**
Particle size and RDI%.

| Formulation | Particle Size (nm) | RDI % |
|---|---|---|
| Ziprasidone Nanosuspension | 252 | – |
| Spray Dried Nanosuspension | 267 | 105.9 |
| Freeze dried Nanosuspension | 280 | 111 |

Particle size and redispersibility index (RDI %) of spray dried and freeze dried nanosuspensions. The RDI% of spray dried nanosuspension is close to 100 while it is higher in the case of freeze dried nanosuspension which may be because in spray dried nanosuspension spherical shaped particles are formed and nanocrystals are present on the surface while in the case of continuous matrix freeze dried nanosuspension the structure of the nanocrystals (according to SEM study) is similar to that of spray dried nanosuspension. as seen and in this matrix nanocrystals (according to SEM study) are present so minimum aggregation of nanoparticles is present in spray dried nanosuspensions as compared to freeze dried nanosuspensions and therefore % RDI of spray dried nanosuspensions is less as compared to freeze dried nanosuspensions.

## Saturation Solubility

The saturation solubility of pure Ziprasidone was found to be 4.5Mg/ml whereas in case of nanosuspension, spray dried nanosuspension and lyophilized nanosuspension it was found to be 45 Mg/ml, 43 Mg/ml and 40 Mg/ml respectively. About 8-fold increase in saturation solubility was observed in Ziprasidone nanosuspension, spray dried nanosuspension and lyophilized nanosuspension.

## *Differential Scanning Calorimetry (DSC)*

Differential scanning calorimetry studies were carried out to evaluate the drug-excipient compatibility and to check the possible changes in crystallinity of the drug on milling process of the media. DSC thermograms of pure ziprasidone, Vitamin E TPGS and Physical mixture are depicted in Figure 8 while ziprasidone, mannitol, spray dried nanosuspension and lyophilized nanosuspension are shown in Figure 9. Vitamin E TPGS showed an endothermic peak at 42°C and pure ziprasidone showed an endothermic peak at 303°C while DSC thermogram of physical mixture showed two endothermic peaks at 42°C and 304°C.

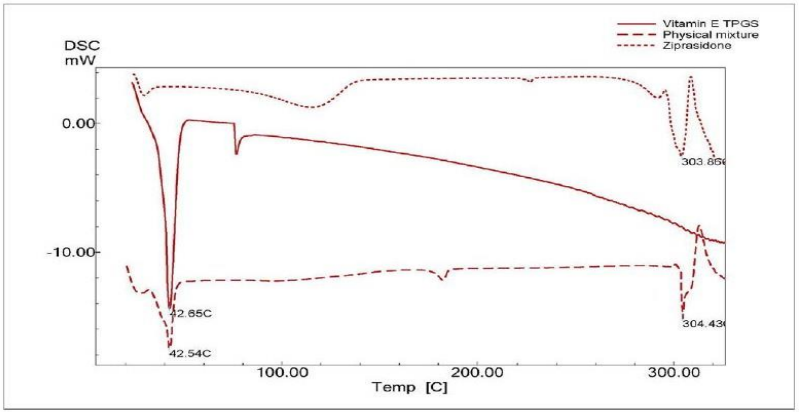


**Figure 8**. DSC thermograms of pure Ziprasidone, Vitamin E TPGS and Physical mixture.

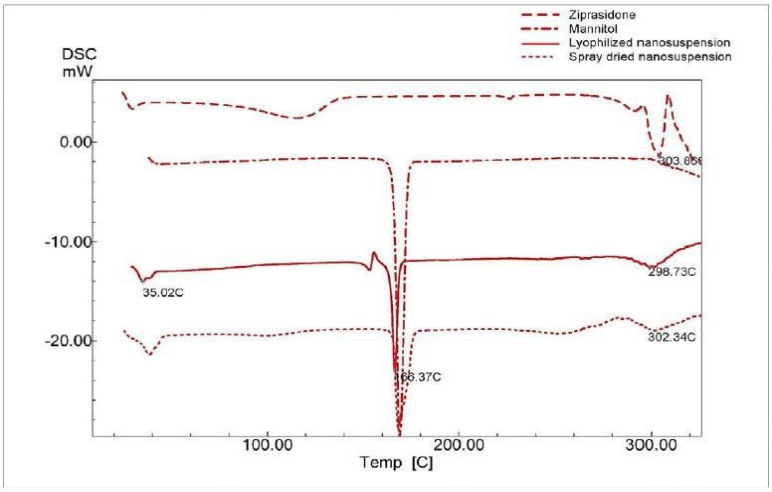


**Figure 9**. DSC thermograms of Ziprasidone, mannitol, spray dried and lyophilized nanosuspensions.

The characteristic endothermic peaks of vitamin E TPGS and pure ziprasidone were retained in the physical mixture indicating the compatibility of ziprasidone with vitamin E TPGS. DSC thermograms of lyophilized ziprasidone nanosuspension showed endothermic peaks at 35°C and 298°C while spray dried ziprasidone nanosuspension showed endothermic peaks at 38°C and 302°C. The characteristic peaks of ziprasidone were slightly sifted to lower temperatures in both lyophilized and spray dried nanosuspensions which may be due to the presence of vitamin E TPGS on the nanoparticle surface moreover an additional peak at 166°C was also seen in both lyophilized and spray dried nanosuspensions which is due to mannitol.

***In Vitro* Dissolution Study**

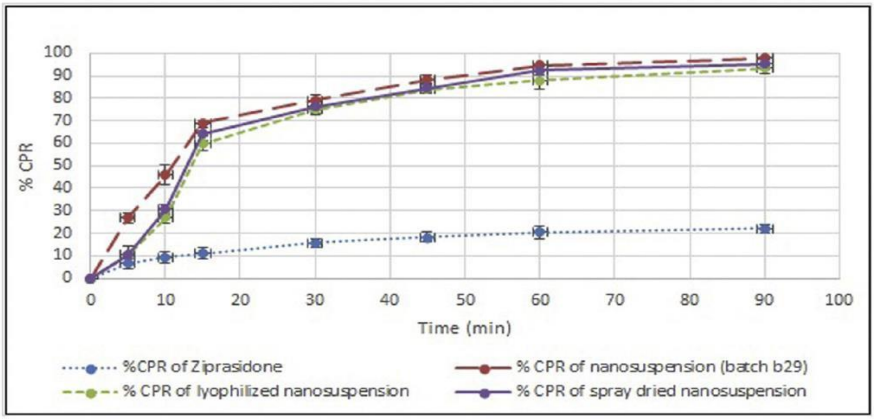


**Figure 10.** Dissolution profiles of pure Ziprasidone, Ziprasidone nanosuspension, spray dried and lyophilized nanosuspension.

Figure 10 shows the dissolution profile of pure ziprasidone, ziprasidone nanosuspension, spray dried nanosuspension and lyophilized nanosuspension. Ziprasidone showed poor dissolution characteristics and only about 20% of the drug was released within 90 minutes whereas it was more than 90% in the case of ziprasidone nanosuspension, spray dried nanosuspension and lyophilized nanosuspension. The dissolution efficiency at 45 min was calculated and the order was ziprasidone nanosuspension > spraydried nanosuspension > lyophilized nanosuspension > ziprasidone (63% > 57% > 55.8% > 12%). This increase in nanosuspension dissolution rate can be explained from the Noyes Whitney equation. At the beginning of 15 minutes dissolution of spray dried nanosuspension and lyophilized nanosuspension was less compared to Ziprasidone nanosuspension which may be due to the dissolution of matrix and further release of soluble Ziprasidone nanocrystals.

**X-ray Diffraction Study**

X-ray diffraction studies were carried out to confirm the crystalline state of spray dried Ziprasidone nanosuspension and lyophilized Ziprasidone nanosuspension. XRD patterns of Vitamin E TPGS, pure ziprasidone, spray dried ziprasidone nanosuspension and lyophilized ziprasidone nanosuspension. From the diffrectogram results it was observed that there is no difference in the peak position of pure ziprasidone in spray dried and lyophilized ziprasidone nanosuspensions which indicates that lyophilized and spray dried ziprasidone nanosuspensions are in the same crystal form as pure ziprasidone. The presence of TPGS vitamin E and high energy input during milling did not change the crystalline state of ziprasidone. However a slight decrease in peak intensity was observed in lyophilized ziprasidone nanosuspension which may be due to reduction in particle size and crystallinity.

**Scanning Electron Microscopy (SEM)**

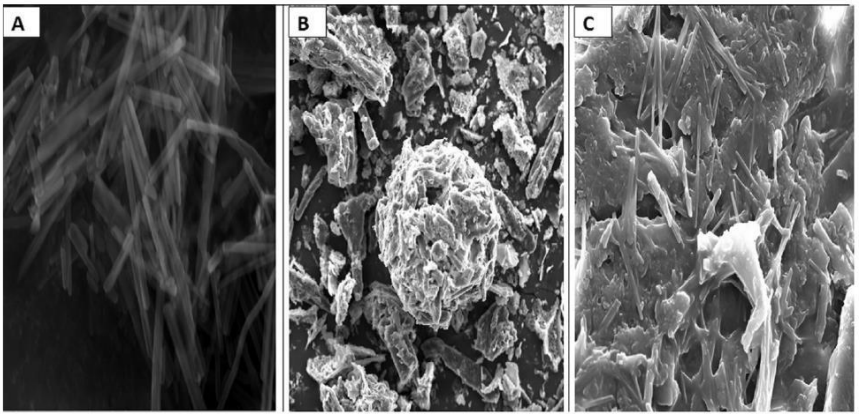


**Figure 12.** Scanning Electron Microscopy dari A) Ziprasidone nanosuspension, B) spray dry nanosuspension dan C) Lyophilized Ziprasidone nanosuspension.

Ziprasidone nanosuspension, spray dried nanosuspension and lyophilized nanosuspensions are shown in Figure 12. Ziprasidone nanosuspension shows almost rod shaped crystals whereas in spray dried nanosuspension almost spherical shaped particles are observed and on the surface of these spherical particles, rod shaped nanocrystals are seen. In the matrix of lyophilized nanosuspension such as mannitol structure is seen and in this matrix rod shaped nanocrystals are dispersed. So mannitol in lyophilized nanosuspension prevents aggregation of nanocrystals.

## CONCLUSIONS

Improved solubility of Ziprasidone nanosuspension has been successfully achieved through the application of media milling technique. The study found that wet media milling technique, followed by drying, has significant potential to improve the solubility and dissolution of the drug. The next step in this study was to optimize the process parameters and formulation of the media milling technique. To achieve this, a careful preliminary study was conducted to determine the optimal conditions for the study. Analysis of the particle characteristics, particularly the particle size of Ziprasidone, was the main focus in this optimization process. The results showed that the physical stability of the nanosuspension that had been optimized using spray drying and lyophilization techniques was quite good. Various parameters indicate the possibility of aggregation in very small nanosuspension particles, but the promising results of this study provide a strong foundation for improving the effectiveness and applicability of Ziprasidone in medicine.

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