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Original Article

FORMULATION, OPTIMIZATION AND CHARACTERIZATION OF ZIPRASIDONE NANOCRYSTALS PREPARED BY MEDIA MILLING TECHNIQUE

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ABSTRACT

Objective: Today, nanotechnology has a variety of application areas. Pharmacy is one of the most important application fields of nanotechnology. Preparation of nano-particular drug-delivery system, such as nano-crystals improve the solubility and bio-availability of poorly water soluble drugs.

Methods: Ziprasidone (ZIP) is a low water soluble drug (Bio-pharmaceutical classification system) and is used as a lipid regulating agent. In this study, a rapid and simple media milling method was used for the preparation of ziprasidone (ZIP) Nanocrystals. The use of sonication after media milling process reduced the milling time significantly. Different concentrations of stabilizers (Kollidone and Tween 80) were tested in the preparation of ZIP nanocrystals. The finest ZIP nanocrystals were obtained by 4 g ZIP, 4 g kollidone and 4 g Tween 80.

Results: The size and zeta potential of the finest ZIP nanocrystals were 238.2±2.5 nm and-19.6±0.1 mv, respectively. The morphology of dried ZIP nanocrystals was observed using scanning electron microscopy. Differential scanning calorimetry of ZIP and ZIP nanocrystals confirmed that there was no interaction between ZIP and stabilizers. Compared with ZIP, the solubility of ZIP nanocrystals increased significantly.

Conclusion: Media milling technology was successfully used for the formulation of poor water soluble drugs. Nano-sized drug particles prepared by media milling technique could improve the solubility and bio-availability of those drugs.

Keywords: Ziprasidone, Nanocrystals, Poorly soluble drugs, Media Milling.

INTRODUCTION

Approximately, 40% of newly discovered drugs show poor solubility in water [1]. The poor water solubility of drugs causes its poor bio-availability [2]. There are many approaches to improve the solubility of poorly water soluble drugs. Some of these approaches include salt formation of drugs, use of co-solvents, surfactants and complexing agents [3]. Moreover, it has been reported that particle size reduction of drugs can increase the solubility of drugs [4]. Today, nanotechnology is an important tool to increase the solubility of poor water soluble drugs. The particle size reduction of these drugs to the nanometer range can improve their dissolution rate and bio-availability because of an increase in their surface area and saturation solubility [5, 6].

Media milling is an efficient technique used to reduce the particle size of drugs to nanometer range and increase their water solubility. In this technique, the drug particles in aqueous suspension are size-reduced by grinding using small and hard beads. The drug particle size is reduced by the attrition of drug particles with the grinding media. Nano-sized particles tend to form aggregates as a result of their large surface area. In order to minimize drug particle aggregation, it is necessary to stabilize drug particles using stabilizers, such as polymers and surfactants. The stabilizers adsorb on the surface of drug particles and stabilize the drug particles through ionic interactions [7]. Ziprasidone is a serotonin dopamine antagonist that works to treat the positive, negative and depressive symptoms associated with schizophrenia. The positive symptoms include visual and auditory hallucinations and delusions. The negative symptoms, which are harder to treat, include blunted affect, social withdrawal, and lack of motivation [8].

The aim of this work was to prepare ZIP nanocrystals using a modified media milling method to increase its water solubility. In this work, we used a combination of sonication and media milling method for the preparation of ZIP nanocrystals. This combination reduced the processing time of media milling. The optimized nanocrystals of ZIP were characterized by particle size and size distribution, differential scanning calorimetry (DSC) and Fourier transform infrared (FTIR) spectroscopy.

MATERIALS AND MEHODS

Ziprasidone and Tween 80 (polyoxyethylene sorbitan monooleate) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Kollidone was purchased from Fluka (Germany). All other materials used were of analytical grade.

Preparation of ziprasidone nanocrystals

For the preparation of stabilizer solution, Tween 80 was dissolved in purified water and then kollidone was added under mechanical agitation. Pure ZIP was dispersed into the stabilizer solution and kept under mechanical agitation. Once a uniform suspension was formed, the suspension was placed into a glass tube. Zirconium beads (two mm in diameter) were also placed into a glass tube. Nanocrystals were prepared after milling the suspension using zirconium beads. The tubes were shaken using an IKA® orbital shaker (Vibrax VXR Basic, Germany) with 1500 rpm at room temperature for 75 min. Then, milled suspension was sonicated using an ultrasonic bath at 37 °C for 30 min. Different concentrations of stabilizers were tested in the preparation of the finest ZIP nanocrystals and then spray dried. The details about the formulation design are described in table 1.

Evaluation

Particle size

Particle analysis was performed by using the Malvern instruments. Before measurement, the samples had to be diluted with de-ionized water to obtain a suitable concentration for measurement. Ten micro-liter of nanocrystals were diluted with 1 ml purified water [9].

Zeta potential (particle charge)

Zeta potential was determine using Malvern Zeta Sizer to estimate the physical stability of nano suspension. The sampleswere prepared using de-ionized water with conductivity adjusted by

addition of sodium chloride before measurement. All measurements were performed in triplicate [10].

Differential scanning calorimetry (DSC)

Thermal properties of ZIP and ZIP nanocrystals were analyzed using a DSC-1 (Mettler Toledo, Switzerland). Approximately, 5 mg of samples was placed in aluminum pans. The measurements were carried out at temperatures from 20 °C to 250 °C at a scan rate of 20 °C/min.

Scanning electron microscope (SEM)

The Nanocrystals were examined under a Scanning Electron Microscope. The morphology of the Ziprosidone Nanocrystals was examined by scanning electron microscopy (JSM 6390 India). The sample was mounted on an aluminum stub and sputter-coated for 120 s with platinum particles in an argon atmosphere. The coated samples were then scanned, and the image was analyzed at 500 or 1000 axis.

Fourier transforms infrared

Pure ZIP, Pure ZIP with kollidone, Pure ZIP with tween and ZIP nanocrystals was diluted with potassium bromide and made into pellets. The FTIR spectra of samples were recorded using an FTIR spectrometer.

Determination of solubility for ziprasidone (ZIP) and ZIP nanocystals

The aqueous solubility of ZIP and ZIP nanocrystals was determined by a shake-flask method. Briefly, an excess amount of ZIP and dried ZIP nanocrystals were suspended in 9 ml of phosphate buffer pH 7.5, and the suspensions were shaken at 37 °C. Aliquots of solutions were withdrawn and filtered through a 0.22 µm whatman filter. The concentration of ZIP was determined in filtrates using Thermo UV/Visible Spectrophotometer (Genesys, USA) at 276 nm [15].

Table 1: Formulation design of ZIP nanocrystals using different concentrations of stabilizers

Formulation	Ziprasidone (g)	Tween (g)	Kollidone (g)	Water (ml)
F1	1.6	1.6	1.6	65
F2	4	4	4	100
F3	4	2	2	115
F4	4	4	8	115
F5	4	4	4	115

Fig. 1: Pure ziprasidone and ziprasidone nanocrytals

***In vitro* drug release studies**

In vitro drug release studies were performed into USP Type 2 dissolution apparatus (Electrolab, India) using basket method at rotation of 75 rpm. The dissolution was carried in acid media. The volume and temperature of the dissolution medium were 900 ml and 37±0.2 °C, respectively. The samples were withdrawn at fixed times and were filtered and assayed through ultraviolet absorbance determination at 315 nm using a UV–visible spectrophotometer. The mean result of triplicate measurements, and the standard deviation wasreported [13].

RESULTS

As shown in table 1, several formulations of ZIP nanocrystals were prepared using different concentrations of Tween 80 and kollidone as stabilizers. Drug concentration was fixed at 0.5%.

Particle size

Among the different concentrations of stabilizers, the finest ZIP nanocrystals (F2) were obtained by 4 g kollidone and 4 g Tween 80. Particle size of F2 was 238.2±2.5 nm.

Zeta potential

Zeta potential of F2 was found to be 0.465±0.05 mv

Differential scanning calorimetry (DSC)

Fig.4 and Fig.5 show DSC thermo-grams of pure ZIP and the finest ZIP nanocrystals [F2] respectively. The pure ZIP showed a typical endothermic peak at 60.91^{0}c, which corresponds to its melting point and the finest ZIP nanocrystals[F2] showed an endothermic peak at 55.8^{0}c.

Scanning electron microscopy

The SEM image of the ziprasidone nanocrystals revealed that the particles are crystalline in shape.(Fig.1 and 3). The average size of the Ziprasidone nanocrystals was found to be less than 300 nm.

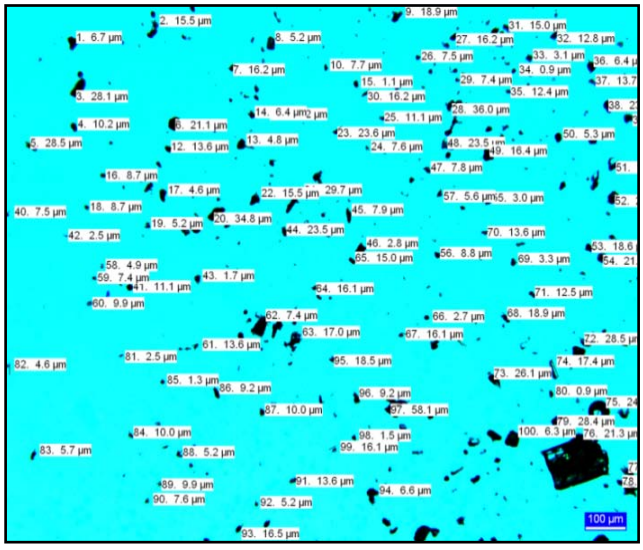

Fig. 2: particle size of prepared ziprasidone nanocrystals

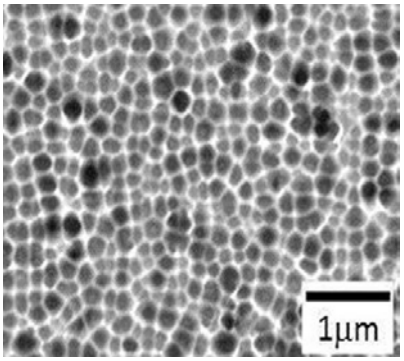

Fig. 3: SEM image of the ziprasodone (ZIP) nanocrystals

Fourier transform infrared

The molecular states of ZIP and the finest ZIP nanocrystals (F2) were investigated using FTIR. fig. 5 shows the FTIR spectrum of the ZIP and the dried finest ZIP nanocrystals (F2) in the range of 400-4000 cm $^{-1}$.

The spectrum of the dried finest ZIP nanocrystals (F2) as shown in Fig.6, show no obvious difference with the ZIP spectra in the whole area of ZIP absorption bands.

Solubility for ziprasidone (ZIP) and ZIP nanocrystals

The solubility of ZIP in buffer was 1.0±0.01 µg/ml while the solubility of ZIP from the dried finest ZIP nanocrystals (F2) was 8.2±0.03 µg/ml. Therefore, solubilty of the dried finest ZIP NC (F2) was found to be almost 8 times higher , as compared to that of the parent ZIP.

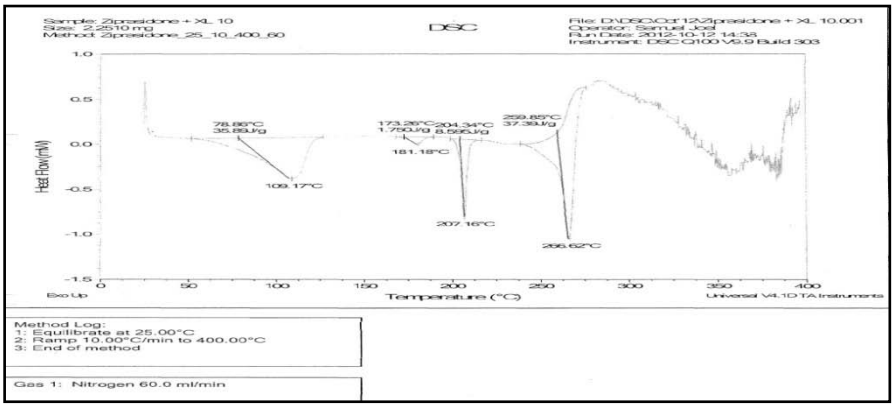

Fig. 4: Differential scanning calorimetry thermo-gram of bulk ZIP powder

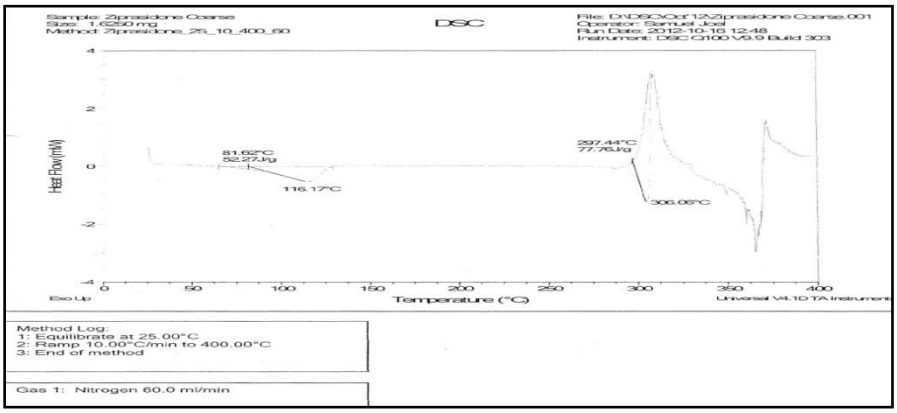

Fig. 5: Differential scanning calorimetry thermo-gram of the finest Ziprasidone (ZIP) nanocrystals (F2)

DISCUSSION

Recently, the media milling technology is successfully used for the formulation of poorly water soluble drugs. The nano-sized drug particles prepared by media milling technique can improve the solubility and bio-availability of these drugs [12,13]. However, the main drawback of this technique is its longer processing time [14]. Many researchers have attempted to reduce the processing time by different strategies. For example, drug particle size reduction using a jet mill before media milling process can reduce the processing time of media milling [15]. The new combinational methods have also been developed for particle size reduction. These combinational methods can overcome the limitations of conventional size particle size reduction technologies and reduce the drug nanocrystals production time. In this study, we have used sonication to reduce the processing time of media milling for the preparation of ZIP nanocrystals. We have already prepared ZIP nanocrystals (mean particle size: 245±12 nm) by media milling method using Tween 80 and kollidone as stabilizers after 24 h milling time. The use of sonication after media milling process has also reduced the milling time from 24 h to 75 min. As shown in table 1, the finest ZIP nanocrystals have (F2) shown mean particle size of 238.2±2.5 nm.

The zeta potential of the finest ZIP nanocrystals (F2) is around-20 mV, which is enough for sufficient electrostatic stabilization of ZIP nanocrystals. [16]. The morphology and size of the finest ZIP nanocrystals (F2) have been evaluated by SEM. DSC technique to investigate the effect of stabilizers on the ZIP structure. Comparing the DSC thermograms of ZIP and ZIP nanocrystals, no significant differences has been found between ZIP and the finest ZIP nanocrystals (F2) curves. It has been concluded that there was no interaction between ZIP and stabilizers. The shift of ZIP peak in the

ZIP nanocrystals sample to the lower temperature can be due to the presence of Tween 80 on the surface of ZIP nanocrystal [7]. The FTIR spectra of pure ZIP and ZIP nanocrystals have confirmed the presence of ZIP in the ZIP nanocrystals. Moreover, from the results of FTIR study, it can be concluded that there is no evidence of chemical interaction between ZIP and stabilizers. During solubility study, it has been observed that the solubility ZIP nanocrystals has been increased up to be 8-fold due to the formation of ZIP nanocrystals.

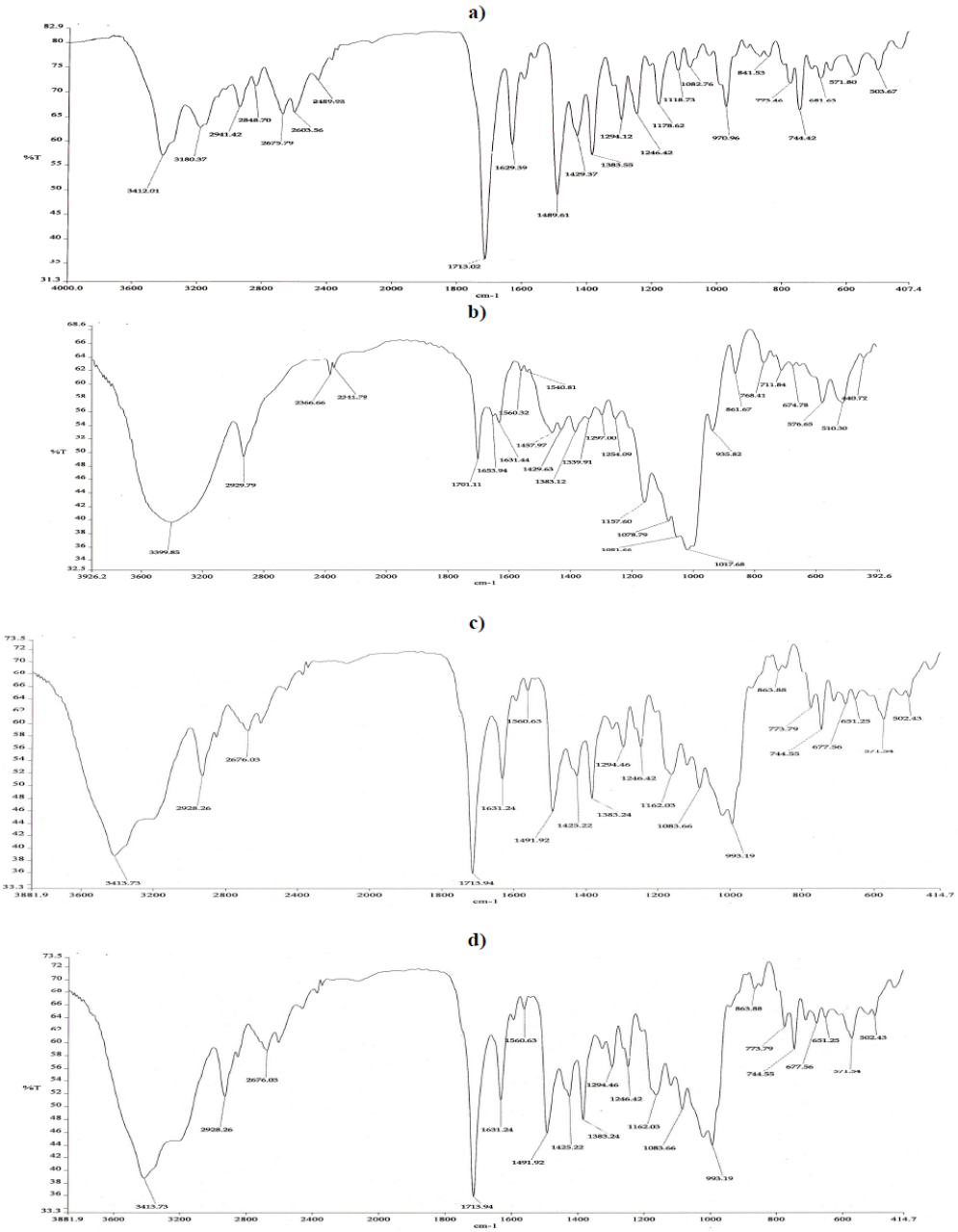

Fig. 6: (a) FTIR of ZIP alone (b) FTIR of ZIP with tween (c) FTIR of ZIP with kollidone (d) FTIR of ZIP nanocrystals

In vitro drug dissolution

The results of the in vitro drug dissolution studies have been depicted in Table 2 and Fig.7.

Table 2: *In vitro* drug release from prepared ZIP nanocrystals capsules (sample size =3 for each formulation)

Formulation	10 min	15 min	20 min	30 min	45 min	60 min
F1	28±2.1	46±2.6	61±2.6	82±2.5	92±2.2	97±2.1
F2	19±2.5	24±2.3	37±2.5	53±2.2	64±2.5	81±2.0
F3	23±2.3	47±2.4	50±2.0	80±2.4	97±2.2	100±2.3
F4	18±2.1	29±2.1	40±2.2	59±2.7	67±2.5	95±2.5
F5	15±2.4	26±2.6	49±2.6	67±2.2	75±2.3	99±2.4

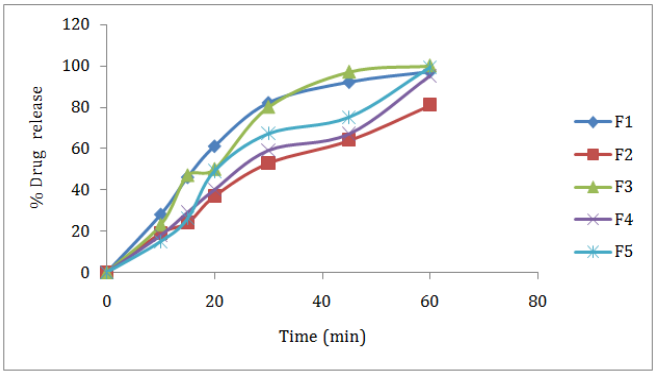

Fig. 7: *In vitro* dissolution profile of prepared ZIP nanocrystals capsules (sample size =3 for each formulation)

CONCLUSION

In this study, nanocrystals of a poorly soluble drug ZIP were prepared easily using a combination of wet milling method and sonication. These nanocrystals could be used for the preparation of a promising new drug formulation of ZIP. Solubility study showed that ZIP nanocrystals gave higher solubility as compared to pure ZIP. Consequently, The ZIP nanocrystals could be a promising alternative drug delivery system to improve the bio-availability of ZIP.

CONFLICT OF INTERESTS

Declared none

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