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Production and In Vitro Characterization of Solid Dosage form Incorporating Drug Nanoparticles

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The objective of this study was to develop a tablet formulation of ketoconazole incorporating drug nanoparticles to enhance saturation solubility and dissolution velocity for enhancing bioavailability and reducing variability in systemic exposure. The bioavailability of ketoconazole is dissolution limited following oral administration. To enhance bioavailability and overcome variability in systemic exposure, a nanoparticle formulation of ketoconazole was developed. Ketoconazole nanoparticles were prepared using a media-milling technique. The nanosuspension was layered onto water-soluble carriers using a fluid bed processor. The nanosuspensions were characterized for particle size before and after layering onto water-soluble carriers. The saturation solubility and dissolution characteristics were investigated and compared with commercial ketoconazole formulation to ascertain the impact of particle size on drug dissolution. The drug nanoparticles were evaluated for solid-state transitions before and after milling using differential scanning calorimetry (DSC) and powder X-ray diffraction (PXRD). This study demonstrated that tablet formulation incorporating ketoconazole nanoparticles showed significantly faster rate of drug dissolution in a discriminating dissolution medium as compared with commercially available tablet formulation. There was no affect on solid-state properties of ketoconazole following milling. The manufacturing process used is relatively simple and scalable indicating general applicability to enhance dissolution and bioavailability of many sparingly soluble compounds.

Keywords nanosuspension; poorly soluble drugs; particle size; ketoconazole; dissolution

INTRODUCTION

Ketoconazole is an imidazole antifungal agent suitable for the treatment of candidiasis and other systemic fungal infections. The major drawback in the therapeutic application and efficacy of ketoconazole as an oral dosage form has been its very low aqueous solubility (0.017 mg/mL) because of its hydrophobic structure (Connors & Elder, 2004; Van der Meer et al., 1980). The low solubility across the physiological pH range of ketoconazole is reported to result in erratic and variable absorption from the gastrointestinal (GI) tract, hence not recommended for use in treating life-threatening fungal infections (Levine, 1982). On the basis of its solubility across physiologically relevant pH conditions and absorption characteristics, ketoconazole is classified in the Biopharmaceutics Classification System (BCS) as a class II drug. Although the compound has good permeability (108 ± 18 nm/s, Caco-2), the solubility in GI fluids is insufficient to dissolve the administered dose under normal conditions (Balimane, Han, & Chong, 2006; Galia et al., 1998).

Formulation approaches that have been reported for dissolution rate enhancement of ketoconazole include solid dispersion with polyvinylpyrrolidone K25 (Van den Mooter et al., 2001), hot-melt extrusion with hydroxypropyl cellulose (HPC) and polyethylene glycol (PEG) (Mididoddi & Repka, 2007), and inclusion complexes with beta-cyclodextrin and hydroxypropyl betacyclodextrins (Esclusa-Diaz, Gayo-Otero, Perez-Marcos, Vila-Jato, & Torres-Labandeira, 1996). However, these approaches have met limited success. Several other general techniques have been developed to enhance the dissolution rate of poorly water-soluble drugs such as ketoconazole. Such methods include solubilization by salt formation. The solubilization technique by salt formation is a complicated process and is not feasible for a compound that does not have ionizable groups.

An approach that is commonly used to increase dissolution velocity and impact saturation solubility of sparingly soluble compounds such as ketoconazole is to formulate it as nanometer-sized particles, particles usually less than 1 μm in diameter. For example, when the particle size of the drug is reduced from

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8 μm to 200 nm, there is 40-fold increase in the surface area to volume ratio. This increase in surface area can provide substantial increase in the dissolution rate if the formulation disperses into discrete particles (Liversidge & Cundy, 1995).

Nanocrystalline dispersion or nanosuspension comprises drug, water, and stabilizers. Stabilizers used to aid the dispersion of particles are either polymers and/or surfactants. To be effective, the stabilizers must be capable of wetting the drug crystals and providing steric and ionic barrier. In the absence of appropriate stabilizers, the high surface energy of the nanometer-sized particles would tend to agglomerate or aggregate the drug crystals. Too little of stabilizers can cause aggregation and too high can cause Ostwald ripening. The concentration of polymeric stabilizers can range from 1 to 10% (wt/vol), and the concentration of surfactants is generally <1% (wt/vol) (Liversidge, Liversidge, & Cooper, 2003).

Nanocrystalline dispersions or nanosuspension at high concentrations (>20% wt/vol) suitable for granulating or spray drying can be prepared using the media mill. The manufacturing can be described as a simple procedure comprising attrition media, suspension, and agitation. The extent of size reduction by wet bead milling is governed by amount of grinding energy, which is determined by the intrinsic hardness of the drug, grinding media, and milling power. Using this process, nanocrystalline dispersion at concentrations up to 40% (wt/vol) can be prepared easily (Liversidge et al., 2003).

In this study, media milling was evaluated for the production of ketoconazole nanosuspension. The drug nanoparticles were assayed for dissolution and solubility behavior to confirm theoretical enhancement predictions. Solid-state transitions were also evaluated before and after particle size reduction. Conversion of nanosuspension into solid intermediate was achieved by layering on to a water-soluble carrier such as lactose. The recovery of particles from granules was assessed following layering. Use of water-soluble carrier prevents particle agglomeration in powder state and further increase dissolution characteristics (Hecq et al., 2006).

MATERIALS AND METHODS

Materials

Ketoconazole was purchased from Halycon laboratories (Mumbai, India). Lactose monohydrate (Tablettose 70®) was purchased from Meggle GmbH (Wasserburg, Germany). Hydroxypropyl methylcellulose (HPMC, 6 cps) was purchased from Colorcon (Mumbai, India). Sodium lauryl sulfate (SLS) was purchased from Qualigen chemicals (Delhi, India). Polyvinyl pyrolidone (PVP K30) and Poloxamer 188 were purchased from BASF (Ludwigshafen, Germany). Crospovidone (polyplasdone) and sodium starch glycolate (SSG) were purchased from ISP (USP) and Grain processing corporation (Muscatine, IA, USA), respectively. All other chemicals were of analytical grade.

Methods

Preparation of Nanosuspension on a Laboratory Scale

A laboratory scale in-house glass apparatus was used to identify suitable stabilizer for preparing nanosuspensions. The glass apparatus mimicking the media-milling machine was fabricated in-house. The apparatus comprised a double-walled jacketed cylinder having a volume of 250 mL. In this process, the drug substance was dispersed uniformly in an aqueous medium containing dissolved stabilizers in the milling chamber using a Heidolph mixer (Model: RZR2051 Control, Rose Scientific Ltd., Alberta, Canada) operated at 500 rpm. The solid content of the suspension was 5 or 10% (wt/wt). The milling media comprising 0.2-mm yttrium-stabilized zirconium beads and additional water were added into the milling chamber. The total volume of slurry (drug substance + stabilizer + water) was 100 mL. The batch size for these development trials was 100 mL, and the temperature of the suspension was maintained at 20–25°C during milling by circulating cold water. The milling media was agitated using a Heidolph mixer operating at a speed of 1600 rpm. The milling time was fixed at 7 h. Following milling, the beads were filtered, and the nanosuspension was collected and stored at refrigerated conditions (2–8°C) until further use. Generally, the choice and concentrations of stabilizer not only depend on its ability to facilitate particle size reduction but also its ability to produce suspensions with acceptable physical stability. Typically, a combination of steric and electrostatic stabilization is most effective for nanosuspensions' stability (Liversidge et al., 2003). The composition of surfactant and polymeric stabilizers used for production of ketoconazole nanosuspension is summarized in Table 1. The stability of the nanosuspension was evaluated upon storage at refrigerated and room temperature conditions for a period up to 1 week.

Particle Size Analysis

Particle size and size distribution of the suspension before, during (at different milling times), and following milling were determined using a laser diffraction (LD) method, with a wet sampling system (Mastersizer S, Malvern Instruments, Worcestershire, UK). The particle diameters reported were calculated using volume distribution. The median volume particle size, d50 (size of the particles for which 50% of the sample volume contains particles smaller than "d50," the other particles being larger than "d50"), d10, and d90 were used as characterization parameters. A refractive index of 1.5 was used for measurements.

The particle size obtained with the different stabilizer compositions and their physical stability upon storage is summarized in Table 2. On the basis of particle size distribution obtained following milling and suspension stability, a formula composition comprising HPMC as the primary stabilizer and SLS as the secondary stabilizer (Formulation NS8) were chosen for scale-up trials using the media-milling machine.

TABLE 1
Formula Composition of Ketoconazole Nanosuspensions

Formulation Code	Formulation Composition (%, wt/vol)					Batch Size (mL)
	Drug	SLS	HPMC (6 cps)	Poloxamer 188	PVP-K30	
NS1	5.00	0.75	1.00	–	–	100
NS2	5.00	0.75	–	1.00	–	100
NS3	5.00	0.75	–	–	1.00	100
NS4	5.00	0.25	2.00	–	–	100
NS5	5.00	0.50	2.00	–	–	100
NS6	5.00	0.75	2.00			100
NS7	5.00	0.75	3.00	–	–	100
NS8	10.00	0.75	2.00	–	–	100
NS9*	10.00	0.75	2.00	–	–	5000
MS1	10.00	0.75	2.00	–	–	100

NS1–8: Nanosuspensions manufactured by in-house fabricated apparatus using commercial drug substance, particle size (d90)–110 µm.

MS1: Microparticulate suspension manufactured using micronized drug substance, particle size (d90)–5.22 µm.

NS9: Scaled-up batch manufactured by Netzsch bead mill using micronized drug substance.

Scale-Up of Manufacturing Process for Production of Nanosuspension

The selected formula composition (Formula NS8) was scaled-up using a bead mill (Model: Lab Star 1, Netzsch mill, Netzsch, Germany). The milling chamber was charged with the milling or grinding media. The milling media comprised 0.2-mm yttrium-stabilized zirconium beads. The milling operation was performed in a re-circulation mode with the suspension fed at a rate of 150 mL/min. The mill and pump speeds were operated at 2500 and 120 rpm, respectively. The suspension flowed axially through the milling chamber where the shear forces generated during impaction of the milling media with the drug provided the energy input to fracture the drug crystals into nanometer-sized particles. The temperature inside the milling chamber was controlled by circulating cooling water through the outer jacket. After milling, the suspension was filtered and stored at a temperature below 25°C until further processing.

Conversion of Nanosuspensions into Solid Intermediate by Fluidized Bed Processes

The spray-layering process (Model: GPCG 1.1, Fluidized Bed Coater, Glatt GmpH, Germany) was used to obtain solid intermediate as granules for tabletting. The formula composition of suspension (NS9) used for layering on to a water-soluble carrier is detailed in Table 3. The nanoparticulate or microparticulate suspensions were layered at a spray rate of 6 g/min on to fluidized lactose particles in the product container of fluid bed coater. The amount of lactose monohydrate used was 151.5% (wt/wt) with respect to ketoconazole content before the spray-layering operation. The drying temperature and atomization speed were set at 40–45°C and 1.2 bar, respectively. The granules were dried as they moved upward in the airflow. Small droplets and low viscosity of the spray medium ensured that distribution was uniform. The particle recovery from granules following layering is summarized in Table 4.

Dissolution Studies

Dissolution rates of ketoconazole were determined according to a method described by Nogami, Nagai, and Yotsuyanagi (1969) with minor modifications. A USP dissolution apparatus (Model: DISSO 2000, Labindia, Mumbai, India) type II (paddle method) with a paddle operating at 50 rpm was used for dissolution studies. All dissolution tests were carried out on an equivalent of 200 mg of ketoconazole (in suspension and powder state) and tablets. Acetate buffer, pH 4.5, was used as dissolution medium. The volume and temperature of the dissolution

TABLE 2
Particle Size of Nanosuspension Prepared on Laboratory Scale

Formulation Code	Particle Size Distribution (µm)								
	Initial			1 week @ 25°C			1 week @ 2–8°C		
	d10	d50	d90	d10	d50	d90	d10	d50	d90
NS1	0.090	0.213	0.655	0.091	0.215	0.662	0.091	0.215	0.650
NS2	0.098	0.327	2.445	0.118	0.406	2.658	0.099	0.320	2.450
NS3	0.114	0.287	0.921	0.320	0.884	2.502	0.112	0.285	0.918
NS4	0.097	0.247	0.896	0.116	0.262	0.902	0.108	0.258	0.901
NS5	0.090	0.235	0.674	0.091	0.211	0.678	0.089	0.216	0.678
NS6	0.091	0.214	0.606	0.089	0.211	0.607	0.093	0.201	0.602
NS7	0.154	0.673	2.465	0.158	0.681	2.622	0.150	0.675	2.501
NS8	0.063	0.164	0.485	0.070	0.162	0.482	0.061	0.162	0.492

TABLE 3
Formulation NS9: Composition of Solid Intermediate[a]

Ingredients	Quantity/Tablet (mg)
Layering suspension (Nanosuspension)	
Ketoconazole	200.00
SLS	15.00
HPMC (6 cps)	40.00
Purified water qs to	2 mL
Water soluble carrier	
Lactose monohydrate	303.00
Total	558 mg

[a]Nanosuspensions was layered onto lactose monohydrate to obtain dry powders.

medium were 500 mL and 37°C, respectively. Samples were withdrawn at predetermined time intervals, filtered in-line, and assayed using an HPLC method (Waters Alliance HPLC system, Milford, MA, USA). Chromatographic separation was accomplished using a Zorbax SB C_{18} 250 × 4.6 mm 5 µm stainless steel column (Agilent Technologies, Foster City, CA, USA). The mobile phase consisted of a solvent acetonitrile and buffer (tetrabutyl ammonium hydrogen sulfate) mixture (25:75% vol/vol). The mobile phase was pumped isocratically at a flow rate of 1.0 mL/min during analysis and was maintained at a column temperature of 25°C. The amount of drug dissolved at each sampling time point was monitored at a UV wavelength of 223 nm.

Saturation Solubility

Saturation solubility evaluations were carried out in buffer media at different pH conditions using a shake flask method. In this method, excess amount (100 mg/mL) of drug substance ("as is" and dried suspension containing microparticles or nanoparticles) was added to 25 mL of each buffer maintained at 37°C and shaken for a period up to 24 h. The samples were filtered using 0.1 µm Millex-VV PDVF filters (Millipore Corporation, Billerica, MA, USA) before analysis. Samples were diluted and concentrations were determined using an HPLC method described earlier. The mean values of triplicate measurements and the standard deviation are summarized in Table 5.

Tablet Preparation

Drug-layered granules were mixed with extra granular excipients in a double cone blender, for 10 min. The formula composition of tablet blend is summarized in Table 6. The blend was compressed into tablets using a mini press compression machine (Model: Mini-II B, Rimek, Ahmedabad, India) fitted with a 9-mm round, flat-faced tooling. The physical properties of tablets, hardness, friability, and disintegration time, were measured and are summarized in Table 7. The tablet hardness was measured using a hardness tester (Model: 8M, Dr Schleuniger Pharmatron, Manchester, NH, USA). Each hardness value reported is an average of ten measurements. The disintegration time was measured in purified water at 37 ± 0.5°C, using a disintegration tester (Model: ED2L, Electrolab, Mumbai, India), using sintered disks. The disintegration time reported is an average of six measurements. Tablet friability was calculated as the percentage weight loss of 20 tablets after 100 rotations using a friabilator (Model: EF2, Electrolab).

Solid-State Characterization

Differential Scanning Calorimetry (DSC). Thermal properties of powders were investigated using a Perkin-Elmer DSC-7 differential scanning calorimeter/TAC-7 thermal analysis controller with an intracooler-2 cooling system (Perkin-Elmer Instruments, Waltham, MA, USA). About 3–5 mg of product was placed in perforated aluminum-sealed 50-µL pans, and the heat runs for each sample was set from 40 to 200°C at 5°C/min, under an inert environment using nitrogen. The apparatus was calibrated using indium/cyclohexane.

Powder X-ray Diffraction (PXRD). PXRD diffractograms of commercial and milled ketoconazole formulations were recorded using a Panalytical Xpert Pro Diffractometer (PANalytical, JB Eindhoven, The Netherlands) with a Cu line

TABLE 4
Comparisons of Particle Size of Nanoparticles in Suspension and after Layering onto Lactose (Solid Intermediate) by Fluidized Bed Process

Particle Size Distribution (µm)	Formulation NS9		Formulation MS1	
	Suspension	Solid Intermediate	Suspension	Solid Intermediate
d10	0.063	0.065	0.254	0.265
d50	0.121	0.126	0.424	0.482
d90	0.242	0.239	5.224	5.924

Formulation NS9: Nanosuspension.
Formulation MS1: Suspension with micronized drug substance.

TABLE 5
Saturation Solubility of Commercial, Micronized, and Dried Nanoparticle Ketoconazole

	Solubility (mg/mL) (Mean ± *SD*)		
Solvents	Unmicronized[a] Ketoconazole	Micronized[a] Ketoconazole	Dried Ketoconazole Nanoparticles[b]
0.1 N HCl	1.22 ± 0.08	1.76 ± 0.12	58.53 ± 4.86
Acetate buffer pH 4.5	0.21 ± 0.01	0.34 ± 0.06	0.48 ± 0.06
Water	0.00	0.10 ± 0.01	0.41 ± 0.07

[a]Solubility was tested in respective solvents containing HPMC and SLS, 2 and 0.75% (wt/vol), respectively.

[b]Nanoparticles were containing HPMC (2.0%, wt/vol) and SLS (0.75%,wt/vol).

TABLE 6
Composition of Tablets

	Formula Composition (mg/tablet)			
Ingredients	NST1	NST2	NST3	MST1[a]
Spray Layered Granules[b]	558.00	558.00	558.00	558.00
Sodium Starch Glycolate	80.00	–	–	–
Corn Starch	–	80.00	–	–
Crosspovidone	–	–	80.00	80.00
Colloidal Silicon Dioxide	6.00	6.00	6.00	6.00
Magnesium Stearate	6.00	6.00	6.00	6.00
Total (mg)	650.00	650.00	650.00	650.00

[a]NST1–3: Tablet formulation contains nanosized ketoconazole; MST1: Tablet formulation contains micronized ketoconazole.

[b]Spray-layered powder containing 200 mg ketoconazole, 15 mg SLS, 40 mg HPMC, and 303 mg lactose monohydrate.

TABLE 7
Physical Properties of Tablet Formulations

Formulation Code	Hardness (Kp)	DT (min)	Friability (%)
NST1	11.0 ± 0.24	20 ± 2.4	0.09
NST2	10.3 ± 0.46	>20	0.02
NST3	9.6 ± 0.42	10 ± 1.2	0.03
MST1	10.8 ± 0.44	9 ± 1.0	0.06

as the source of radiation. Standard runs using a 40-kV voltage, a 40-mA current, and a scanning rate of 0.02° min^{-1} over a 2θ range of 3–40° were used.

RESULTS AND DISCUSSION

Selection of Stabilizer Composition for Production of Physically Stable Nanosuspensions

The formula compositions evaluated with different polymeric stabilizers (NS1–NS3) showed differences in particle size distribution (Table 2). The median particle sizes (d50), for the milled suspensions for the three formula compositions (NS1, NS2, and NS3), were 0.213, 0.327, and 0.287 μm, respectively. The d90, which is indicative of large particles or aggregates, were 0.655, 2.445 and 0.921 μm. On the basis of the particle size distribution obtained for the three formula compositions upon milling for a predetermined time interval, HPMC was selected as the primary stabilizer for steric stabilization of ketoconazole nanoparticles. The better stabilization with this polymer may be attributed to its adsorption characteristics based on affinity as compared with PVP and poloxamer. It also leads to the smallest final particle size among the stabilizers investigated (Table 2). Although poloxamer 188 has been shown to be successful in the stabilization of nanosuspensions, their use has been limited because of its low melting point that may pose processing problems during conversion to solid intermediates either using a fluid bed process or using spray drying. Similar observation was made by Hecq et al. (2006), when comparing HPMC with polyvinyl alcohol, poloxamer 188, and acacia for stabilizing UCB-35440-3, a sparingly water soluble weak base that has a large dose.

The effect of SLS concentration (0.25, 0.5, and 0.75%, wt/vol) on particle size distribution of nanosuspension indicated no effect on d10 and d50. However, the d90 value obtained decreased with increasing SLS concentration, were 0.896, 0.674, and 0.606 μm at 0.25, 0.5, and 0.75% (wt/vol) SLS concentrations, respectively. An effective particle size reduction was observed when SLS concentration was 0.75% (wt/vol).

The effect of HPMC concentrations (1 & 2%, wt/vol) on particle size distribution of nanosuspension obtained indicated that there was no effect on the median particle size. The median particle size (d50), for formulation composition with 1 and 2% (wt/vol) HPMC concentrations were 0.213 and 0.214 μm, respectively. However, formulation containing HPMC 3% (wt/vol) had a median particle size (0.673 μm). Similar trend was also obtained for large particles (d90). Surprisingly, low concentration of polymeric stabilizer was required to form stable nanosuspensions as reported by other investigators (Shah et al., 2006). This could be attributed to the fact that higher concentration of polymers would result in increased viscosity that could hinder particle attrition at same milling energy (Hecq, Deleers, Fanara, Vranckx, & Amighi, 2005). A formulation containing 2% (wt/vol) HPMC was selected as it provided the right balance for stabilization without affecting viscosity required for effective milling.

The physical stability of nanosuspension was evaluated following storage in refrigerated (2–8°C) and room temperature conditions (25°C) for a period up to 1 week. The particle size

distribution of nanosuspension before and after storage for the different formula compositions is detailed in Table 2. There was no significant change in particle size distribution of nanosuspension incorporating HPMC and Poloxamer as the primary stabilizer. However, in PVP-stabilized nanosuspensions, an increase in particle size was observed, which is more pronounced at higher temperature (25°C). This could be attributed to the fact that heterogeneity in particle size distribution could result in difference in saturation solubility between particles. These differences could have resulted Ostwald ripening leading to crystal growth with concomitant increase in particle size (Van den Mooter et al., 2001). Ostwald ripening did not occur in suspension containing HPMC and poloxamer as the primary stabilizer for two reasons. In the first instance, the drug candidate is poorly soluble, thus leading to insignificant changes in the dissolved concentration during preparation and storage. Secondly, the particles are relatively homogenous in size, thus avoiding larger differences in the saturation solubility between differently sized crystals (Nystrom, 1998). The effect of suspension concentration or solids content on particle size distribution of nanosuspension at identical processing conditions indicated that the more concentrated suspension showed smaller particle size distribution (Table 2). The concentrated nanosuspensions showed smaller particle size; this might be attributed to increased collision of particles at higher concentrations during processing (Krause & Muller, 2001). Alternatively, smaller particles can also be obtained by increasing the applied total disintegration energy (Krause & Muller, 2001). The nanosuspension obtained with different solids content showed good physical stability upon storage for a week at room temperature and refrigerated conditions. The particle size distribution before and after storage is summarized in Table 2. The primary stabilizer (HPMC) and secondary stabilizer (SLS) used at concentrations of 2 and 0.75%, respectively, were able to maintain the physical stability of the nanosuspension even at higher drug concentration. This stabilizer composition was selected for scale-up using the agitator bead mill. Physical stability of nanosuspensions upon storage for a period up to 3 years has been reported (Peters, 1996); this could be explained based on absence of Ostwald ripening (Jacobs, Kayser, & Muller, 2006). Long-term stability of nanosuspensions were not studied in this case because the prepared nanosuspensions are only intermediate products and were subsequently converted to solid intermediates for solid dosage forms.

Preparation of Nanosuspension Using Bead-Milling Process

The kinetics of particle size reduction using the wet bead-milling process is shown in Figure 1. As reported by other investigators (Lee, 2003), the particle size was found to decrease with time. Particle size reduction generally depends on the fragmentation of drug crystals because of impaction with the milling media (Ploehn & Russel, 1990). Following 3 h of wet milling,

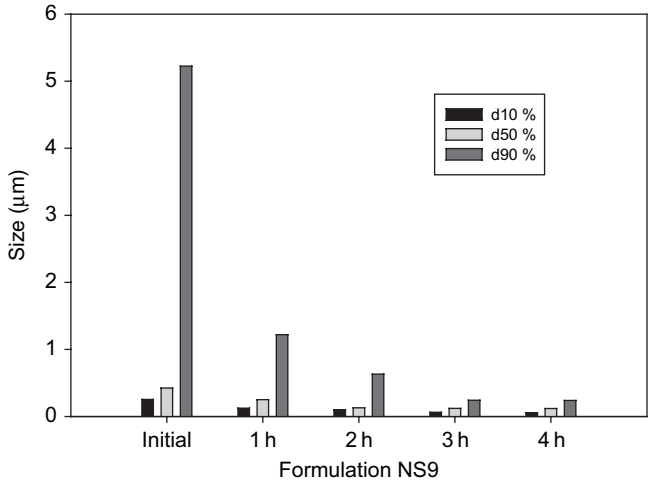

FIGURE 1. Particle size distribution of Nanosuspension NS9 as a function of milling time. (Diameters d10, d50, and d90% in µm). (Stabilizer combination: SLS @ 0.75% and HPMC @ 2.0% and drug content @ 10%).

the particle size reached a plateau value (254 nm), and a Gaussian particle size distribution was observed. The final particle size obtained was dependent on the stabilizers/drug ratio. A Formula composition comprising HPMC and SLS at concentrations of 20 and 7.5% (wt/wt), respectively, relative to ketoconazole content (200 mg) were found optimal in stabilizing the drug nanoparticles (250 nm @ d50). The results from these studies and others indicate that wet bead milling is a versatile technology capable of producing drug nanosuspension at high concentrations (>10% solids content) for poorly soluble drugs (Liversidge et al., 1996, 2003).

Stability of suspension prepared using the bead-milling process was evaluated to reconfirm the stabilizers selected. The nanosuspension with the selected formula composition showed good physical stability following storage for a period up to 4 weeks at room temperature and refrigerated conditions. The particle distribution of nanosuspension following storage is shown in Figure 2. On the basis of our findings and by other investigators, drug nanosuspension can be stabilized using stabilizers at a weight ratio (drug: stabilizer) in the range of 20:1 to 2:1 (Liversidge et al., 2003). Liversidge et al. (2003) reported that for a poorly soluble drug such as naproxen, when the particle size was reduced from 20to 30 µm to the nanometer range (d50–270 nm) following the use of wet milling, the ratio of drug to the stabilizer, PVP (K-15), was 5:3. The naproxen nanoparticles did not aggregate and its physical and chemical stability was maintained for a period up to 4 weeks at 4°C (Liversidge & Conzentino, 1995; Liversidge & Cundy, 1995). In another study, nanosuspension of paclitaxel containing 2% (wt/vol) paclitaxel and 1% (wt/vol) pluronic F127 as stabilizer and prepared using wet bead milling showed that optimal amount of higher molecular weight polymeric

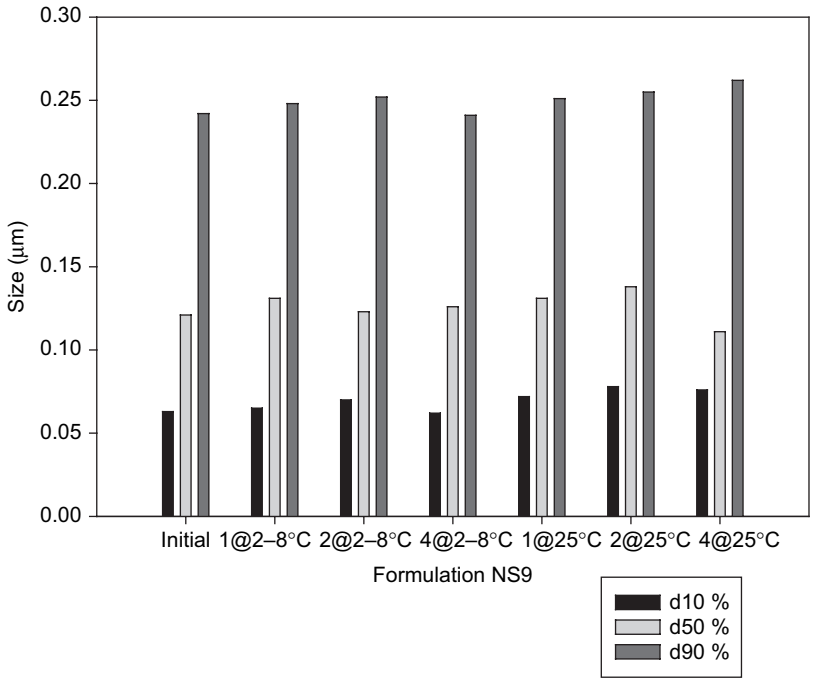

FIGURE 2. Particle size distribution of Nanosuspension NS9 as a function of storage conditions. (Diameters d10, d50, and d90% in µm).

stabilizer was needed for effective particle size reduction and shelf stability (Liversidge et al., 1996, 2003; Liversidge & Cundy, 1995).

The solid-state transitions of ketoconazole following milling studies using DSC and X-ray diffraction techniques indicated that drug crystallinity was maintained and no solid-state transitions were observed. The DSC thermogram of ketoconazole before and after milling is shown in Figure 3. The X-ray diffractograms for the milled and unmilled drug is shown in Figure 4. There are instances where solid-state transitions are observed if processing is not done in a controlled environment (Byrn, Pfeiffer, Ganey, Hoiberg, & Poochikian, 1995). The absence of any solid-state transitions may be attributed to the fact that milling was performed under controlled temperature conditions and the aqueous phase effectively dissipates the heat generated during processing (Liversidge et al., 2003).

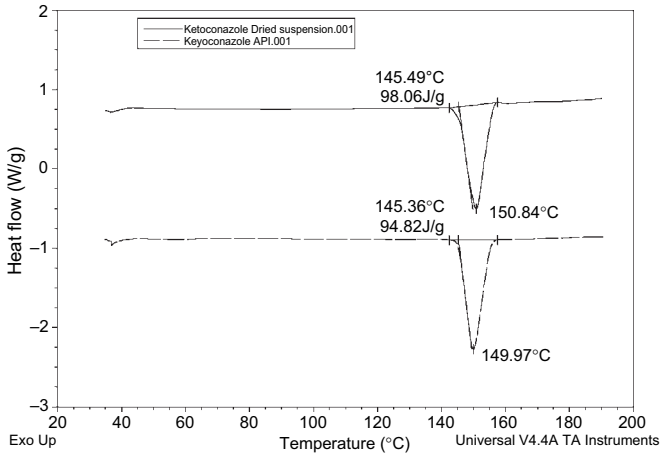

FIGURE 3. DSC thermograms for (dotted line) unmicronized ketoconazole and (solid line) dried ketoconazole nanosuspension. [Drying was done at room temperature (25°C) for 3 days].

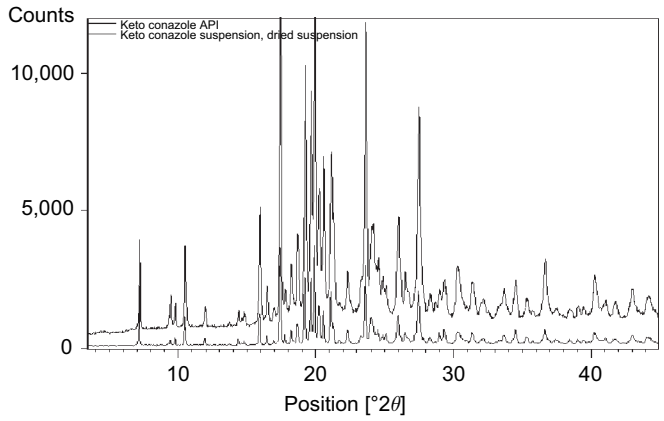

FIGURE 4. PXRD diffractograms for (Bottom) unmicronized ketoconazole and (Top) dried ketoconazole nanosuspension. [Drying was done at room temperature (25°C) for 3 days].

Conversion of Nanosuspensions into Solid Intermediate Using Fluidized Bed Processes

Drying nanosuspensions using conventional processes, such as spray drying and freeze-drying, has been utilized extensively for further processing into various dosage forms (Hecq et al., 2005, 2006; Lee, 2003; Lee & Cheng, 2006). The solid dosage form should be able to quickly disperse into nanometer-sized drug crystals upon dissolution. Otherwise, the surface area enhancement will not be fully utilized to achieve the desired bioavailability. Therefore, a key property of dried nanoparticles is their recovery, that is, whether they can reconstitute to nanometer-sized particles when dispersed in an aqueous medium.

Table 4 summarizes particle size distribution from solid intermediates following re-dispersion in an aqueous medium. The solid intermediates obtained showed good recovery, indicating lactose chosen as the water-soluble carrier for layering prevented agglomeration. (Hecq et al., 2005, 2006). Lactose, because of high aqueous solubility offers the advantage of creating a highly hydrophilic environment around ketoconazole nanoparticles, thus mitigating agglomeration upon dispersion.

Solubility Studies

Saturation solubility of solid intermediates or granules containing drug nanoparticles was evaluated in 0.1 N HCl, acetate buffer pH 4.5, and water (~pH 6.8) at physiological temperature (37°C) and compared with "as is" and micronized drug. The results from these studies summarized in Table 5 indicated a decrease in drug solubility with increasing pH. This observation was found in agreement with Carlson, Mann, and Canafax. (1983). The higher solubility in acidic pH (0.1 N HCl) condition as compared with acetate buffer pH 4.5 or water could be attributed to the weakly basic nature of ketoconazole. The saturation solubility of granules incorporating drug nanoparticles was significantly higher than micronized and un-milled drug at all pH conditions. These results clearly demonstrate that reduction in particle size to sub-micron or nanometer range affects saturation solubility, which may result in enhancement in dissolution velocity and concomitantly higher bioavailability (Liversiedge et al., 2003; Muller, Jacobs, & Kayser, 2001).

Compression into Tablets

The physical properties of the tablets are summarized in Table 7. Three formula compositions with different disintegrants were compressed into tablets with the hardness of 9–11 Kp. The friability of all the tablets was <0.1% indicating good mechanical strength. The disintegration results indicated that among the different disintegrants, crospovidone showed the fastest disintegration (10 min) and cornstarch the slowest disintegration (>20 min). The superior disintegrant property of crospovidone with ketoconazole may be attributed to its nonionic nature. It has been demonstrated that crospovidone, a nonionic disintegrant, shows better disintegration with cationic drugs such as ketoconazole. Other disintegrants such as croscarmellose sodium and SSG being anionic retard dissolution of cationic drugs (*Pharmaceutical Technical Bulletin*, ISPCorp.com).

Dissolution Rate Evaluation

The comparative dissolution of suspension containing drug nanoparticles, micronized and un-micronized drug in acetate buffer is shown in Figure 5. There was a significant increase in the rate of drug dissolution for the nanoparticulate suspension as compared with suspension incorporating micronized and un-micronized drug. The increase in dissolution velocity may be attributed to its smaller particle size and increased surface area (Esclusa-Diaz et al., 1996). The simultaneous increase in saturation solubility and decrease in diffusional distance also leads to increase in dissolution velocity in addition to the surface effect (Muller & Bohn, 1998; Muller et al., 2001).

Formula composition of tablet formulations incorporating nanoparticle and micronized ketoconazole are summarized in Table 6. These formulations had similar physical properties and dimensional characteristics with commercial ketoconazole tablet formulation. The dissolution characteristic of these tablet formulations in pH 4.5 acetate buffer is shown in Figure 6. The rate and extent of drug dissolution from tablets incorporating ketoconazole nanoparticles was significantly higher, 65% as compared with 45% for micronized and 37% for commercial formulation at the end of 60 min. However, the drug dissolution from all formulations was incomplete. This may be explained by the fact that when powder undergoes compression, the re-dispersible spray-dried particles would tend to

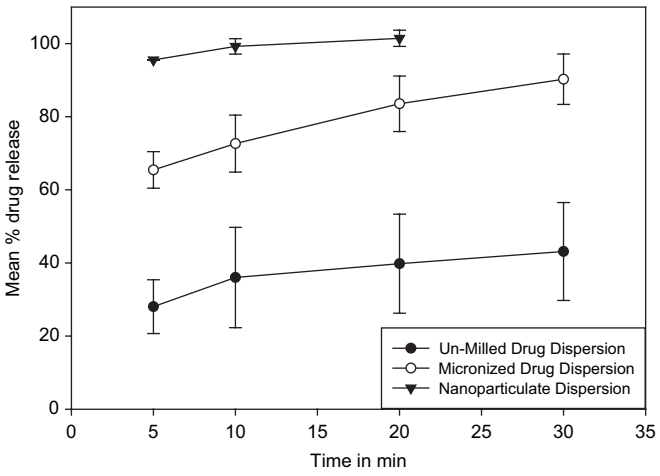

FIGURE 5. Dissolution profile comparison of ketoconazole nanosuspension with suspensions containing micronized and unmicronized ketoconazole. Particle size (d90): unmicronized – 110 μm; micronized – 5.22 μm, and nanosized – 0.24 μm.

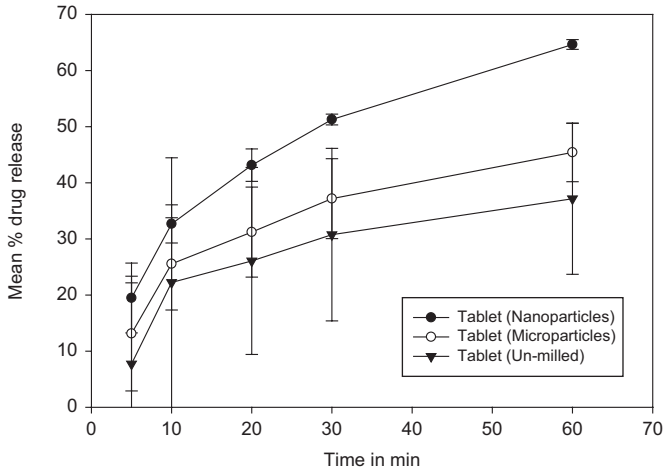

FIGURE 6. Dissolution profile comparison of tablet formulation containing drug nanoparticles (NST3) with tablet formulations containing micronized (MST1) and unmicronized ketoconazole. Particle size (d90): unmicronized – 110 μm, micronized – 5.22 μm, and nanosized – 0.24 μm.

aggregate and/or fuse together to become larger particles with reduced surface area. Hence, the decrease in drug release was observed with tablet formulation, which was not observed in the suspension formulation.

The physical stability and dissolution characteristics of tablets incorporating ketoconazole nanoparticles was studied upon storage at accelerated (40°C/75% RH) and room temperature conditions (25°C/60% RH) for a period up to 3 months. There was no impact of tablet physical properties upon storage. The dissolution characteristics of the tablets were unaffected upon storage, as shown in Figure 7.

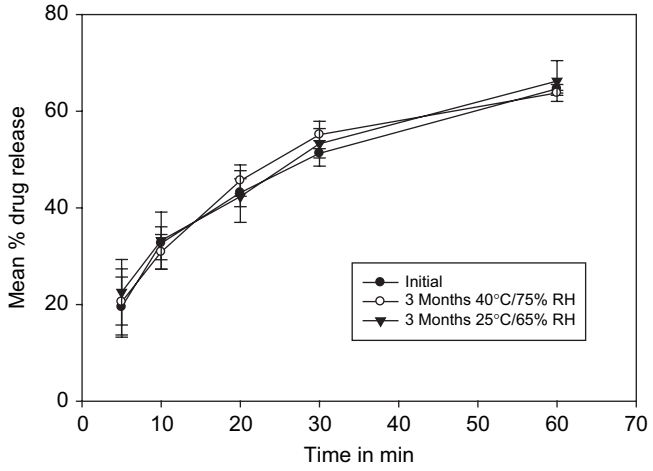

FIGURE 7. Stability evaluation of tablets formulation incorporating drug nanoparticles (NST3) at different storage conditions.

CONCLUSIONS

The use of wet bead-milling technology coupled with fluidized spray-layering process is a viable approach capable of resolving many of the current issues associated with formulation development of poorly water soluble drugs. The approach of reducing particle size to nanometer range in the presence of stabilizers for enhancing oral bioavailability is attractive approach for BCS II compounds (drugs with dissolution rate-limited absorption). The same approach can also be extended to other class of BCS compounds (drugs with dissolution and permeation-limited absorption) if it can be combined with agents that enhance permeation. Enhancing dissolution velocity of sparingly soluble compounds generally correlates with faster absorption rates. The faster absorption rates can correlate into better bioavailability, reduction in fed- and fast effects and inter-subject variability with concomitantly improved therapeutic outcome.

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