ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Development, Physico-Chemical Characterization and Antifungal Activity of Nano-Sized Particles of Posaconazole

Mohd. Imran[1]*, Naira Nayeem[1], and Abdulhakim Bawadekji[2]

[1]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Northern Border University, P.O. Box 840, Rafha 91911, Saudi Arabia.
[2]Deanship of Scientific Research, Northern Border University, P.O. Box 1321, Arar 91431, Saudi Arabia.

ABSTRACT

Posaconazole is a recently Unites States Food and Drug Administration approved antifungal agent. It possesses poor oral bioavailability because of its poor aqueous solubility. The objective of this study was to develop nano-sized particles of posaconazole with the expectation that the nano-sized particles will have better aqueous solubility and, improved antifungal activity. Nano-sizing was done using Supercritical Anti-Solvent (SAS) technology. The average particle size of posaconazole before and after nano-sizing was determined using dynamic light scattering technique. The nano-sized particles were characterized by scanning electron microscopy, FTIR, differential scanning calorimetry, and the powder X-ray diffraction. A USP dissolution apparatus with a paddle operating at 100 rpm was used for the dissolution studies. A marked improvement in the aqueous solubility of posaconazole was observed after nano-sizing, wherein the nano-sized posaconazole showed about 25-fold higher solubility than posaconazole. Screening of antifungal activity was carried out using the well diffusion method, wherein it was observed that the nano-sized posaconazole had improved antifungal activity than posaconazole. It has been concluded that nano-sizing of posaconazole using SAS technology results in posaconazole nano-powder without change in the polymorphic characteristics of the drug, it has improved aqueous solubility, and improved antifungal activity. It is also expected that the nano-sized posaconazole might have improved bioavailability. Therefore, further bioavailability studies are recommended.

Keywords: Posaconazole, Supercritical Anti-Solvent technology, Nano-sized Posaconazole, Aqueous Solubility, Antifungal Activity.

Corresponding author

ISSN: 0975-8585

INTRODUCTION

Posaconazole (PCA, Fig. 1) is a new antifungal triazole derivative, with good tolerability and impressive activity against an extended spectrum of pathogens. It is approved for the treatment of invasive fungal infections, including for the treatment of *aspergillosis*, *chromoblastomycosis*, *coccidioidomycosis*, *fusariosis*, and mycetoma [1,2]. Posaconazole exerts its activity by inhibiting fungal ergosterol biosynthesis, wherein it binds to the heme cofactor located on the target site of lanosterol 14α-demethylase. It has a very low aqueous solubility, which impairs its dissolution in the upper gastric fluid, thus hampering its therapeutic applicability by delaying the absorption rate and thereby onset of action or activity. It possesses poor oral bioavailability (~8%) owing poor aqueous solubility [3]. Together solubility, permeability, and dissolution rate of a drug are essential factors for determining its oral bioavailability. Improvement of aqueous solubility in such a case is a valuable assignment to improve therapeutic efficacy.

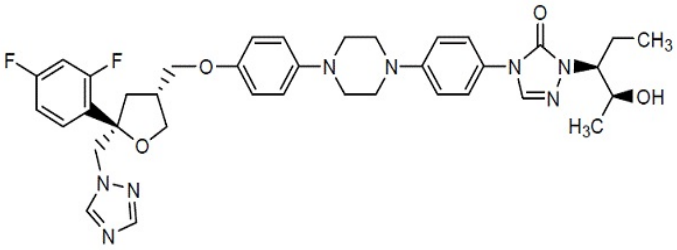

Fig. 1: Posaconazole

The nano-sizing of the drug has been identified as a potentially effective and broadly applicable approach, for example, smaller sized particles have a faster dissolution rate, with potentially higher activity and easier absorption [4-8]. Traditional techniques for particle size reduction such as mechanical milling and precipitation–condensation present considerable success in the nano-sizing of drug particles, but concerns, including broader particle size distribution and the excessive use of organic solvent remain to be addressed [7,8].

In the past decade, supercritical fluid techniques have gained significant attention in many fields, such as chromatography, extraction, organic and inorganic synthesis, porous materials, and material processing for pharmaceutical applications [9–14]. Supercritical fluid, particularly CO_2, present low viscosity, permitting matrix penetration as gas-like characteristic; liquid-like density, promoting solute solubilization; high diffusion; and near-zero surface tension. At the critical point, the density of the gas phase becomes equal to that of the liquid phase, and the interface between gas and liquid disappears. Further, it is nontoxic, nonflammable, and has low price [15].

Particle processing is one of the major developments of supercritical fluid because, besides the novelty related to process characteristics, it also accommodates the principles of green chemistry [14]. Various modified supercritical approaches have been developed [16]. The well-known techniques for particle formation using CO_2 include the rapid expansion of supercritical solutions (RESS) [17] and a variety of anti-solvent processes such as Supercritical Antisolvent (SAS) process [18,19], Gas Anti-solvent (GAS) [20], Particles from Gas-saturated Solutions (PGSS) [21], Aerosol Solvent Extraction Systems (ASES) [22], and Solution-enhanced Dispersion by Supercritical fluids (SEDS) [23]. In the SAS process, the $scCO_2$ and the liquid solution are simultaneously introduced into the high-pressure vessel using nozzle. When the solution droplets reach the $scCO_2$ rapid mutual diffusion at the interface occurs resulting in phase separation and supersaturation leading to nucleation and particle formation. Particle size and shape of the formed particles can be adjusted by means of the process parameter optimization [24]. The objective of this study was to develop nano-sized particles of posaconazole with the expectation that the nano-sized particles will have better aqueous solubility and, improved antifungal activity.

ISSN: 0975-8585

MATERIALS AND METHODS

Materials

Posaconazole (CAS Number 171228-49-2) was obtained from MSN Laboratories Pvt. Ltd, India. All the solvents used were of analytical grade (Merck / SD Fine) and procured from the authenticated and well known chemical suppliers.

Nano-sizing of Posaconazole

Nano-sizing was done using SAS technology supercritical fluid processor (Waters-Thar SFE, Waters, USA) consisting of liquid CO_2 storage tank, CO_2 flow pump, high-pressure mixing vessel, temperature-controlled exhaust nozzle, heat exchanger and collecting vessel. The primary solvent used in feed solution was methanol due to the high solvency power of the PCA in it and due to its high solubility in supercritical CO_2. Briefly, PCA dissolved in methanol was sprayed through the solvent pump into high-pressure vessel pre-filled with CO_2. The effect of the nano-sizing conditions of the solution flow rate (0.1, 0.2 and 0.3 mL/min), pressure (80, 110 and 140 bar), and operating temperature (35, 45 and 55°C) at a constant CO_2 flow rate of 1 kg CO_2/h, was evaluated in respect to the particle size. Once the drug solution was sprayed, solvent rapidly diffuses into the CO_2 and precipitation of drug occurs. Flow of CO_2 was continued for 25 min to allow complete removal of the residual solvent. The resultant drug particles were collected and PCA dispersion was obtained by dispersing particles in water through sonication using bath sonicator.

Particle Size Analysis

The average particle size of PCA before and after nano-sizing was determined using dynamic light scattering (Nano ZS, Malvern Instruments, Malvern UK) at 25ºC. The PCA nano-powder was dispersed in distilled water to form nano-suspension and was then analyzed at an angle detection of 90º. Each value was the average of 3 measurements.

Scanning electron microscopy (SEM) images of PCA before and after nano-sizing were taken using a ZEISS EVO Series EVO 50 (Carl Zeiss International, Germany) microscope operating at an accelerating voltage of 13.52 kV under high vacuum. Freshly prepared samples were fixed to aluminium stubs with double-sided carbon adhesive tape and sputter-coated with conductive gold-palladium.

Thermal properties of PCA 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 the powder was placed in perforated aluminium-sealed 50-L pans and the heat run for each sample was set from 40 to 200ºC at the 5ºC/min, under an inert environment using nitrogen.

Powder X-ray diffraction (PXRD) diffractograms of PCA powder before and after nano-sizing was recorded using a Panalytical Xpert Pro Diffractometer (PANalytical, JB Eindhoven, Netherlands) with a copper line as the source of radiation. Standard runs using a 40-kV voltage, a 40-mA current, and scanning rate of 0.02ºmin^{-1} over a 2θ range of 3–40º were used.

A USP dissolution apparatus (Distek Dissolution system, New Zealand) type II (paddle method) with a paddle operating at 100 rpm was used for dissolution studies. Comparative dissolution profiles were ascertained using 0.1 N HCl pH 1.2 as dissolution medium. The volume and temperature of the dissolution medium was 900 mL and 37 ± 0.5ºC, respectively. Samples were withdrawn at predetermined time intervals, filtered in-line and the amount of drug dissolved at each sampling time point was monitored at a UV wavelength of 262 nm.

Antifungal activity

The antifungal activity of processed and unprocessed PCA was done using strains of *Candida albicans* against which PCA was effective. Potato-dextrose agar medium was used to determine antifungal activity. Screening of antifungal activity was carried out using the well diffusion method [25,26]. One loop full of the

mother-culture was inoculated in 10 ml of agar slant previously in sterilized test tubes, and incubated at 20°C for 48 h. The activity of the processed PCA was compared to unprocessed PCA by determining the zone of inhibition. The drug suspension was prepared using both processed and unprocessed PCA and volume containing 5 µg of each was added to well. Suspensions were then allowed to diffuse into the medium for 2 h by keeping the petri dish at room temperature and then incubated for about 20°C for 48 h. The zone of inhibition was then measured.

RESULTS AND DISCUSSION

Optimization of Nano-sizing Procedure

SAS technology has been widely used for micronization and nanonization of drug particles [18,19], however, there are no reports in literature on nanonization of PCA using this technology. Pharmaceutical technique utilizing SAS can overcome some of the problems associated with dry and wet milling [17]. With this understanding, the objective of the present study was to determine if a nano-milling approach by SAS could be useful in developing an improved formulation of a water insoluble drug, PCA. As a drug delivery platform, nano-sized drug particles have been shown to provide a number of advantages, including enhanced solubility, enhanced bioavailability, improved stability and a delivery platform acceptable for oral administration. Therefore, it was of interest to determine if PCA, being a poorly water soluble, could be processed by SAS technology.

Based on the preliminary findings, the process parameters such as solution flow rate, pressure, and temperature, which are known to influence the size of drug particles were varied to obtain the PCA particles with the smallest particle size. The size of unprocessed PCA and SAS processed PCA are presented in Table 1. The particle size of the unprocessed PCA was found to be 111.2 µm, which were higher than the particles produced by SAS method. Average particle size results presented in Table 1 show a decrease in particle size of PCA with the temperature increase from 35ºC to 55ºC. This could probably be due to increased solubility of PCA at higher processing temperature and that a solubility increase should result in a decrease of nucleation rates and therefore an increase in particle size. This behavior is well explained by several previous studies [29]. According to the values from Table 1, no influence of operational pressure was detected in the particle size. The same behavior was also observed [28] by varying pressures from 80 to 140 bar, using the SAS process with ethanol as primary solvent. From the data presented in Table 1 it was found that with increase in the solution flow rate, an increase in PCA particle size was observed. The possible explanation for this could be that at a higher solution flow rate, the solute solubility is increased in the fluid due to the cosolvent effect of the organic solvent, which promotes slower nucleation kinetics and thus an increase in particle size. Such results were also observed previously during nano-sizing using SAS technique [29]. Finally, the lowest estimated particle size achieved in the present study was obtained at the higher temperature (55°C), at the intermediate pressure (110 bar), at the at lower solution flow rate (0.2 ml/min).

Table 1: Influence of SAS process parameters on particle size of PCA

Parameter		Particle size (nm)
Unprocessed PCA		111.2 µm
Solution flow rate (ml/min)	**0.2**	**325.0 ± 35.5**
	0.4	785.5 ± 110.4
	0.6	985.8 ± 68.9
Operational pressure (bar)	80	358.9 ± 80.4
	110	**325.0 ± 35.5**
	140	340.3 ± 40.8
Temperature (ºC)	35	720.0 ± 50.5
	45	570.7 ± 75.0
	55	**325.0 ± 35.5**

Characterization of PCA nano-particles

SEM micrographs of unprocessed and processed PCA (optimized) are shown in Fig. 2. From the SEM micrograph, it was evident that SAS processing resulted in a significant particle size reduction of PCA.

ISSN: 0975-8585

Unprocessed PCA was large crystalline blocks (Fig. 2A) whereas PCA nano-powder was found to be small sized rod-shaped particles (Fig. 2B). The particle size is in good agreement with that determined by Dynamic Light Scattering (DLS). Further, the polydispersity index (PI) value of 0.19 for the optimized formulation indicates that the particles were uniform.

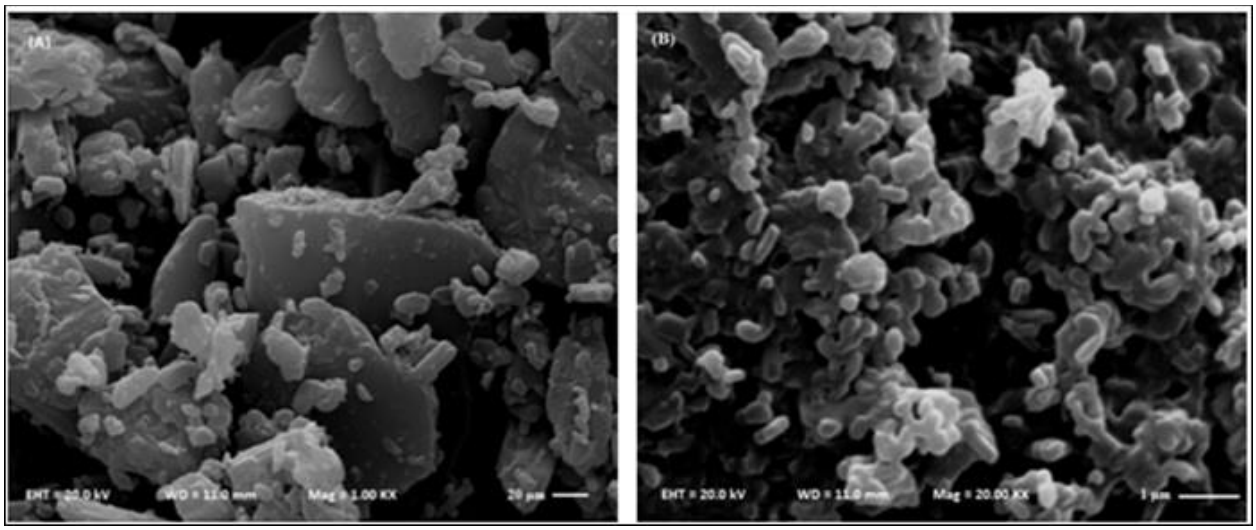

Fig. 2: SEM micrographs of unprocessed and processed PCA (optimized)

The overlapping of the FTIR spectra of unprocessed and processed PCA indicated that there are no changes in functional group after nanonization (Fig. 3). Hence SAS method was good and has not caused any structural changes to the drug. The XRD thermogram (Fig. 4) of processed and unprocessed PCA revealed that the SAS technique does not cause any changes in polymorphic form or crystalline structure of the PCA.

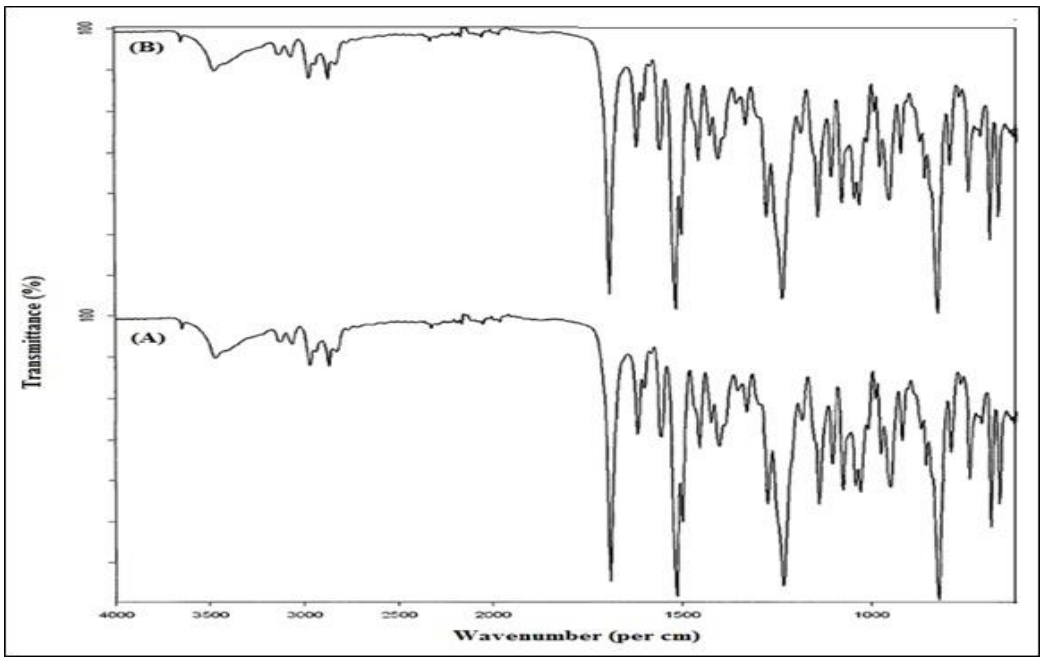

Fig. 3: The overlapping of FTIR spectra of unprocessed and processed PCA

ISSN: 0975-8585

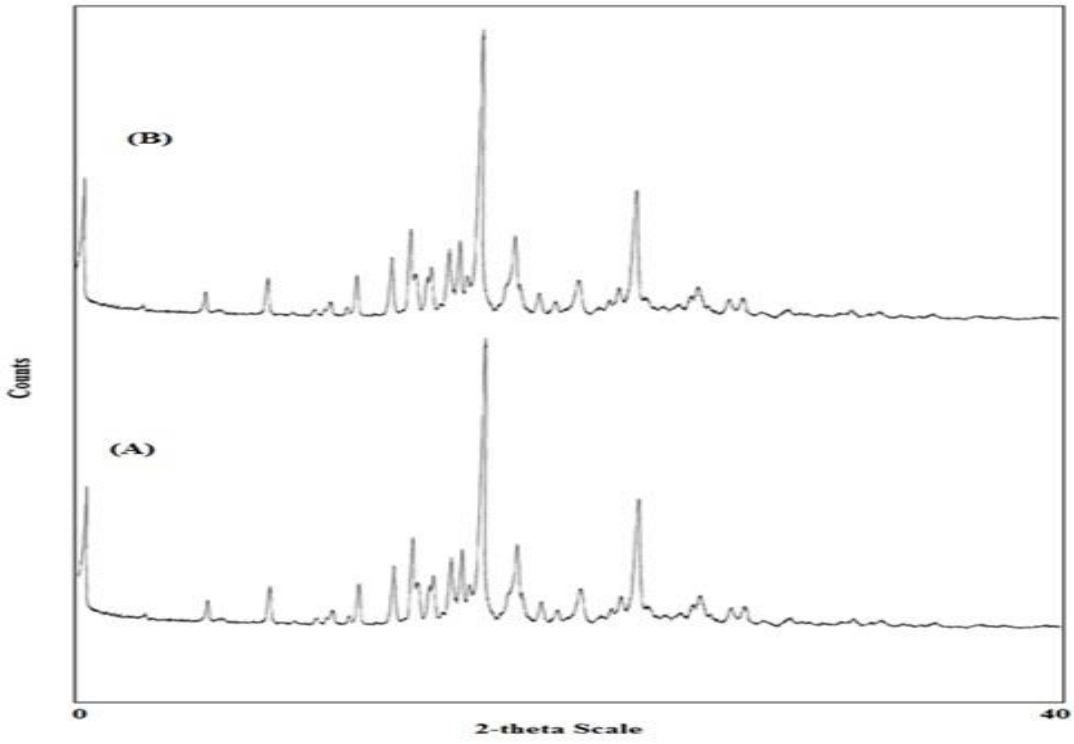

Fig. 4: The XRD thermogram (Fig. 4) of processed and unprocessed PCA

Identical peaks in the thermogram of both processed and unprocessed PCA depicted that nano-sizing technology use in the present work avoids formation of unstable amorphous form of PCA. The same is further proved by DSC spectra since no difference in DSC thermogram can be seen between the processed and unprocessed PCA (Fig. 5).

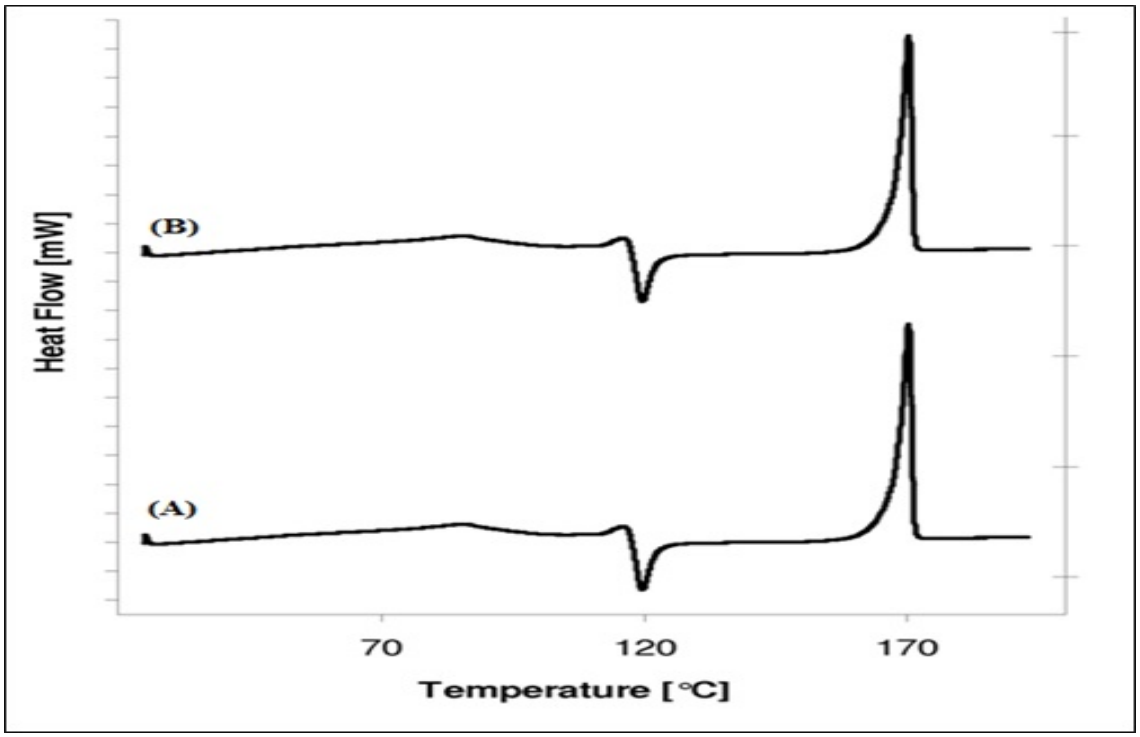

Fig. 5: DSC spectra of the processed and unprocessed PCA

ISSN: 0975-8585

Solubility and dissolution studies

From the solubility data of the unprocessed and processed PCA, it was evident that nano-sizing of PCA has significantly improved its solubility. The aqueous solubility of unprocessed PCA was found to be 0.5 mg/mL. A marked improvement in aqueous solubility of PCA was observed after nano-sizing as the nano-sized PCA has a solubility of 12.3 mg/mL, which is nearly 25-fold higher than that of PCA. Dissolution profile of unprocessed PCA and PCA nano-powder in 0.1 N HCl (pH 1.2) as a dissolution medium is shown in Fig. 6.

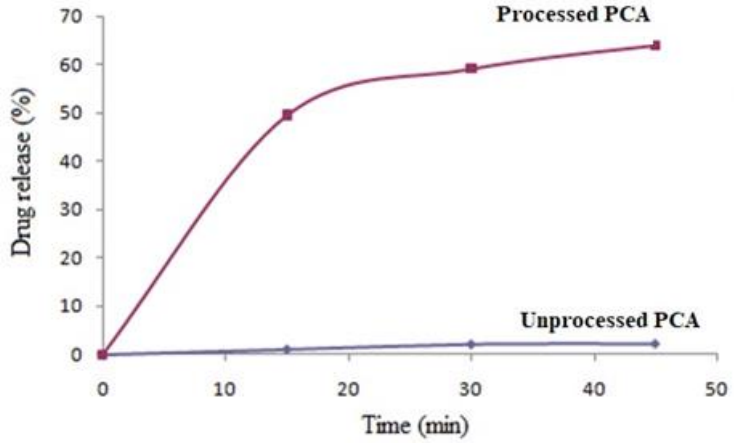

Fig. 6: Dissolution profile of unprocessed PCA and PCA nano-powder

At 15 minute time point dissolution of PCA was approximately 50-fold higher than unprocessed PCA. The complete drug release was observed for nano-powder. The improved dissolution of PCA nano-powder could be due to improved effective surface area owing to nano-sizing. Previous findings also showed that nano-sizing results in improvement in solubility and dissolution [17]. Further, it was reported that the bioavailability of PCA is positively related to its dissolution rate [29] and thus nano-sizing of PCA using SAS technique could result in improvement of bioavailability of PCA.

Antifungal Activity

Maintaining the high biological activity of a drug while developing a novel drug dosage form is the prime objective and hence antifungal activity of PCA nano-powder is evaluated. The activity was observed against *C. albicans* and is compared to PCA suspension. The inhibitory diameter for PCA nano-powder was found to be 28.45 ± 1.13 mm. The values were significantly higher than those obtained for unprocessed PCA suspension (11.50 ± 1.50 mm). The results indicate that the nano-sizing of PCA improves its solubility as well as diffusivity and thus PCA nano-powder diffuses through the agar and causes inhibition of growth of fungi. The PCA suspension has larger size particle which are unable to diffuse since they exist as insoluble entities.

CONCLUSION

Posaconazole is reported to have very low aqueous solubility and poor bioavailability (~8%) [3]. It has been reported that the bioavailability of a drug is positively related to its dissolution rate [29]. Nano-sizing of a drug, by a suitable method like SAS technology, is well recognized method to improve the dug solubility, which ultimately can improve its bioavailability [4-8]. The present study reports that after nano-sizing, posaconazole particles showed approximately 50-fold higher dissolution rate than the unprocessed posaconazole. From the results of the present study, it has been concluded that nano-sizing of posaconazole using SAS technology results in posaconazole nano-powder without change in the polymorphic characteristics of the drug, it has improved aqueous solubility, and improved antifungal activity. Based on the literature related to posaconazole and its analogous drugs, it is expected that the nano-sized posaconazole might have improved bioavailability. Therefore, further bioavailability studies are recommended.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the approval and the support of this research study by the grant no. 7245-PHM-2017-1-8-F from the Deanship of Scientific Research at Northern Border University, Arar, **K.S.A**.

REFERENCES

[1] Nagappan,V, Deresinski S. Clin Infec Dis 2007; 45:1610-1617.
[2] Moore JN, Healy JR, Kraft WK. Expert Rev Clin Pharmacol 2015; 8(3):321-334.
[3] Tang P, Wang L, Ma X, Xu K, Xiong X, Liao X, Li H. AAPS PharmSciTech 2017; 18(1):104-114.
[4] Moghimi SM, Hunter AC, Murray JC. Pharmacol Rev 2001; 53:283-287.
[5] Stolnik S, Illum L, Davis SS. Adv. Drug Deliv Rev 1995; 16:195-199.
[6] Leuner C, Dressman J. Eur J Pharm Biopharm 2000; 50:47-60.
[7] Kayser O, Lemke A, Hernandez-Trejo N. Curr Pharm Biotechnol 2005; 6:3-7.
[8] Pathak P, Meziani MJ, Desai T, Sun Y. J Supercrit Fluids 2006; 37: 279-286.
[9] Cooper AI. J Mater Chem 2000; 10:207-234.
[10] Kikic I, Vecchione F. Curr Opin Solid State Mater Sci 2003; 7:399-405.
[11] Tomasko DL, Li H, Liu D, Han X, Wingert MJ, Lee LJ, Koelling KW. Ind Eng Chem Res 2003; 42:6431-6456.
[12] Nalawade SP, Picchioni F, Marsman JH, Janssen LPBM. J Supercrit Fluids 2006; 36:236-244.
[13] Jiao JX, Xu Q, Li LM. J Colloid Interface Sci 2007; 316:596-603.
[14] Kang Y, Wu J, Yin G, Huang Z, Liao X, Yao Y, Ouyang P, Wang H, Yang Q. , Characterization and biological evaluation of paclitaxel-loaded poly(L-lactic acid) microparticles prepared by supercritical CO2, Langmuir 2008; 24:7432-7441.
[15] Jung J, Perrut M. J Supercrit Fluids 2001; 20:179-219.
[16] Yeo SD, Kiran E. J Supercrit Fluids 2005; 34:287-308.
[17] Debenedetti PG, Tom JW, Yeo SD. Fluid Phase Equilib 1993; 82:311-318.
[18] Chattopadhyay P, Gupta RB. AICHE J 2002; 48:235-244.
[19] Reverchon E, Caputo G, Marco ID. Ind Eng Chem Res 2003; 42:6406-6414.
[20] Gallagher PM, Coffey MP, Krukonis VJ. J Supercrit Fluids 1992; 5:130-142.
[21] Knez Z, Weidner E. Ind Chem Lib 2001; 9:587-611.
[22] Thies J, Müller BW. Eur J Pharm Biopharm 1998; 45:67-74.
[23] Hanna M, York P, Shekunov BY. Proceedings of the 5th Meeting on Supercritical Fluids, France, March 23-25 1998, p. 325.
[24] Adami R, Reverchon E, Järvenpää E, Huopalahti R. Powder Technol 2008; 182:105-112.
[25] Barry AL. Procedures and theoretical considerations for testing antimicrobial agents in agar media. In: Lorian V., Editor. Antibiotics in Laboratory Medicine, 3rd ed. Baltimore: Williams & Wilkins; 1991. p. 1-16.
[26] Varma RS, Khan ZK, Singh AP, Eds. Antifungal agents: past, present, future prospects. Lucknow: National Academy of Chemistry and Biology, India, 1998; pp 55-128.
[27] Martín A, Scholle K, Mattea F, Meterc D, Cocero MJ. Cryst Growth Des 2009; 9:2504-2511.
[28] Mezzomo N, Comim SRS, Campos CEM, Ferreira SRS. Powder Tech 2015; 270:378-386.
[29] Krishna G, Martinho M, O'Mara E. Antimicrob Agents Chemother 2012; 56:4196-4201.