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Original Article

PULMONARY DRUG DELIVERY SYSTEMS OF POSACONAZOLE: DEVELOPMENT AND OPTIMIZATION THROUGH QUALITY BY DESIGN

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ABSTRACT

Objective: The main objective of this work is a formulation of nano-embedded microparticles of posaconazole for targeted delivery into lungs through pulmonary route.

Methods: Posaconazole Nano-Crystals (PNCs) were developed through quality by design approach. Influences of sonication time, sonication power, stabilizer type and its concentration on the solubility and time for 90% drug dissolution (T90%) of the PNC were studied through central composite design. Nanoembedded Microparticles of Posaconazole (NMPs) were developed by nano-crystallization using surfactants to enhance the solubility of posaconazole and then the optimized PNCs were embedded into lactose matrix to enhance the size through lyophilization to obtain NMPs that were suitable for inhalational administration.

Results: The optimized formulation of PNCs was found to exhibit a particle size of 159.2 nm, solubility of 0.29 mg/ml and T90% of 24.7 min. This optimized PNCs were embedded into lactose using lyophilization. The lyophilized product was sieved to obtain NMPs with a size below 5 µm.

Conclusion: The optimized formulation is highly suitable for delivering the drug to lungs through inhalation in which drug particles reach secondary bronchi where they can dissolve readily to yield PNCs. These PNCs can easily escape into alveolar fluids for immediate local action.

Keywords: Posaconazole, Nano-embedded microparticles, Nano-crystallization, Quality by design, Inhalation

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INTRODUCTION

Lungs are the first point of contact with the *Aspergillus* fungi and are going to be infected primarily, with the possibility of spreading to other organs. Antifungals like posaconazole are one of the first-line treatment options available currently [1]. Even after systemic treatment, mortality rates are high, and it is impossible to increase the dose due to their high systemic toxicity. Hence, a drug delivery system that can effectively deliver antifungals to the lungs is more beneficial therapeutically. Posaconazole is insoluble in water and belongs to the BCS Class II category. It is available on the market as an IV injection, an oral suspension, and a delayed-release tablet. The oral products suffer from poor and erratic bioavailability [2]. Considering the limited oral bioavailability, the inhalational route is a more promising and much-needed approach for improved therapeutic efficiency and patient compliance.

Previously, posaconazole co-crystals were developed with adipic acid (1:1) by solvent-assisted grinding, yielding good flowability, a two-fold improvement in solubility, and improved dissolution efficiency of posaconazole [3]. Posaconazoleco-crystals were developed by taking amino acids as co-formers and they found that the formation of co-crystals with L-glutamine [4] and amorphous solid dispersions prepared with Soluplus and PEG 400 resulted enhanced solubility and dissolution rate [5]. These findings altogether demonstrated that the solubility and dissolution rate of posaconazole could be improved by formulating it with hydrophilic carriers. But none of these reports explored the administration of the formulated posaconazole through the pulmonary route. Hence, there is significant scope for exploring the pulmonary route for the administration of posaconazole.

The pulmonary route has substantial advantages, like a very high surface area of the alveoli when compared to the GIT, a lack of first-pass metabolism, comparatively lesser expression of the efflux transporters, and lesser amounts of metabolic CYP450 metabolic enzymes. Besides, the pulmonary route is also highly suitable for the local action of drugs towards air-borne infections like fungal aspergillosis and other pulmonary conditions like asthma, bronchitis, and Chronic Obstructive Pulmonary Disease (COPD). On the other hand, potential barriers to drug administration are also present in this route [6]. Major barriers among them are mucociliary clearance and pulmonary macrophages. Particularly, the mucus present on the ciliary membrane is responsible for this clearance of inhaled particles. Endocytosis of the drug particles by the macrophages is another barrier. This phagocytosis majorly depends on the size and surface properties of the administered drug particles [7]. A drug delivery system that can overcome these two barriers can be the best delivery system for either the local or systemic action of any drug.

Considering the disease etiology and the solubility of posaconazole, the following hypothesis is framed to improve therapeutic efficiency through local delivery in the lungs. Posaconazole Nano-Cystals (PNCs) were planned to enhance the solubility and dissolution rate of posaconazole. But, these particles are unsuitable for pulmonary delivery owing to their poor aerodynamic properties. Hence, these PNCs were aimed to develop into microparticles by embedding in a carrier to obtain Nanoembedded Microparticles of Posaconazole (NMPs) without affecting the posaconazole's dissolution characteristics. These NMPs were expected to have good aerodynamic properties by controlling their size in the range of 1.5 – 5 µm [8, 9]. Henceforth, it is assumed that these NMPs could deposit in the lungs and can be dissolved immediately owing to the high solubility of the carrier so that they can escape pulmonary endocytosis. And upon solubilization, the released PNCs could easily diffuse into the alveolar network for dissolution and local action.

In the present work, PNCs were planned to be developed and optimized though a Quality by Design (QbD) based approach. Several formulation and process variables were optimized to obtain PNCs with maximum solubility and dissolution rate. Later, the optimized PNCs were embedded into a high-soluble lactose matrix using lyophilization. The lyophilized solid was sieved to obtain NMPs with a size below 5 µm with, ensuring unaffected dissolution characteristics of PNCs.

MATERIALS AND METHODS

Materials

Posaconazole was obtained as a gift sample from Intas Pharmaceuticals Ltd., Poloxamer 188, Brij 58 and Lactose was purchased from Merck Ltd.

Table 1: Combinations of the factors with their levels for preparing PNCs

Formulation code	Levels of the factors			
	A: Sonication power	B: Sonication time	C: Stabilizer conc	D: Stabilizer type
PPNC1	50	20	0.08	PLX-188
PPNC2	40	15	0.25	PLX-188
PPNC3	60	15	0.25	PLX-188
PPNC4	40	25	0.25	PLX-188
PPNC5	60	25	0.25	PLX-188
PPNC6	50	11.59	0.5	PLX-188
PPNC7	33.18	20	0.5	PLX-188
PPNC8	50	20	0.5	PLX-188
PPNC9	66.82	20	0.5	PLX-188
PPNC10	50	28.41	0.5	PLX-188
PPNC11	40	15	0.75	PLX-188
PPNC12	60	15	0.75	PLX-188
PPNC13	40	25	0.75	PLX-188
PPNC14	60	25	0.75	PLX-188
PPNC15	50	20	0.92	PLX-188
BPNC1	50	20	0.08	Brij 58
BPNC2	40	15	0.25	Brij 58
BPNC3	60	15	0.25	Brij 58
BPNC4	40	25	0.25	Brij 58
BPNC5	60	25	0.25	Brij 58
BPNC6	50	11.59	0.5	Brij 58
BPNC7	33.18	20	0.5	Brij 58
BPNC8	50	20	0.5	Brij 58
BPNC9	66.82	20	0.5	Brij 58
BPNC10	50	28.41	0.5	Brij 58
BPNC11	40	15	0.75	Brij 58
BPNC12	60	15	0.75	Brij 58
BPNC13	40	25	0.75	Brij 58
BPNC14	60	25	0.75	Brij 58
BPNC15	50	20	0.92	Brij 58

PPNC: Poloxamer188-Posaconazole Nano-crystals; BPNC: Brij 58-Posaconazole Nano-crystals; PLX-188(poloxamer-188)

Development of PNCs

Experimental design

PNCs were planned to be developed by nanocrystallization using the ultra-sonication method. This was carried out through QbD approach [10] with the help of Design Expert software. From the literature and also from the preliminary studies, four different process and formulation factors were selected to study their influence on the solubility and dissolution of posaconazole from the PNCs. Those factors were as follows – A: Sonication power (at 40%, 50%, and 60% amplitude), B: Sonication time (at 15, 20, and 25 min.), C: Stabilizer concentration (at 0.25, 0.5, and 0.75% w/v), and D: Stabilizer type (Poloxamer 188 and Brij 58). On the other hand, solubility and time for 90% dissolution (T90%) of posaconazole from the PNCs were taken as the responses. The central composite design (CCD) was taken as the experimental design to investigate the influences of the factors on the responses. The combinations of the factors with their levels according to the CCD for the development of PNCs are displayed in table 1.

Preparation of PNCs

PNCs were prepared by the ultra-sonication process [11]. The stabilizer at the required concentration was dissolved in 20 ml of distilled water. 100 mg of posaconazole was dispersed in the above stabilizer solution. This dispersion was kept for sonication using a Probe sonicator (Vibra Cell VCX 130, Sonics) at the required amplitude for a specified period of time. The stabilizer, its concentration, sonication amplitude, and time were taken as per the combinations given in table 1. After sonication, the obtained nanosuspension of the PNCs was centrifuged at 8,000 rpm for 10 min. (RM-12C, Remi). Then the solid pellet of the PNCs was collected after removing the supernatant. The obtained pellet was resuspended in double-distilled water and dried by lyophilization. The dried PNCs were stored in the same vial for further studies. The PNCs prepared with Poloxamer 188 (PLX-188) were named PPNCs, and those prepared with Brij 58 were named BPNCs.

Characterization studies of the PNCs

Solubility

Solubility of all the formulations of the PNCs, along with pure posaconazole in distilled water, was determined using equilibrium shake-flask method.

Design of experiments (DoE) analysis

The sequential model sum of squares (SMSS) analysis was performed for each response to identify a suitable regression model with which the factors influenced each response [12]. Later, the suggested model was tested by ANOVA to confirm its validity and significance [13]. Finally, optimization was performed using desirability functions approach. The desirability criteria were set for the responses as maximizing the solubility and minimizing the T90%. Besides, constraints were set for the Factors A and B to as minimize both the sonication power and time, considering the economic aspects of the process.

Particle size and zeta potential

The optimized formulation of the PNCs was subjected to dynamic light scattering using Horiba SZ-100 for determining particle size and zeta potential of the optimized PNCs [14]. This study was performed to ensure the nano-size of the so-called PNCs.

Preparation of NMPs

Lactose was used as the carrier to embed the PNCs. 1 g of lactose was dissolved in 5 ml of water in a glass vial to produce a saturated lactose solution. 0.5 g of the optimized PNCs were added to this lactose solution in the vial and agitated to produce a homogenous dispersion. This dispersion was subjected to lyophilization (LYB-5504, Operon) to obtain a porous matrix of lactose with the PNCs embedded inside [15]. The porous matrix was scraped off to collapse the structure. Later, it was subjected to sieving through a Filson 5 µm stainless steel mesh to yield micronized powder, which was called NMPs.

Characterization of the NMPs

The obtained NMPs were studied by scanning electron microscopy (SEM) [16], differential scanning calorimetry (DSC) [17], and micromeritic characteristics, including mass median aerodynamic diameter (MMAD) [18]. Also, the NMPs were subjected to dissolution studies to investigate any changes in the dissolution rate owing to the lyophilization process.

Dissolution studies

Dissolution studies for the prepared PNCs were conducted in the USFDA-suggested medium, i. e., 900 ml of 0.3% w/v sodium lauryl sulfate [5]. The study was carried out for a period of 1 h with the paddle apparatus set at 25 rpm. Dissolution samples were taken periodically, diluted suitably, and quantified spectrophotometrically. The obtained data was treated kinetically with zero-order and first-order models. Then the dissolution rate constant (k) and T90% were calculated.

RESULTS AND DISCUSSION

PNCs were developed by nano-crystallization using ultrasonication method. The sonication energy could break the particle size of the posaconazole into nano-size. Static charge may be developed due to extreme particle size reduction, and this can cause agglomeration of the nanoparticles. This agglomeration can decrease the effective surface area of the solid and thus result in a negative effect on the solubility enhancement. So as to avoid this agglomeration, a stabilizer was added to the medium during sonication. PLX 188 and Brij 58 were used as the stabilizers. Both of these are hydrophilic non-ionic surfactants that can adsorb on the surface of the posaconazole particles during size reduction. Henceforth, these materials decrease interfacial tension, interfacial free energy, and thus agglomeration [19]. This could finally lead to improved solubility and dissolution rate of the posaconazole. To confirm these assumptions, the prepared PNCs were studied for solubility and dissolution rate, which were also taken as the responses of the study. The obtained results of the solubility and T90% are presented in table 2.

Table 2: Results of solubility and T90% of the prepared PNCs

Formulation code	Value of the responses*	
	R1: Solubility (mg/ml)	R2: T90% (min.)
PPNC1	0.02±0.005	40.4±2.7
PPNC2	0.01±0.006	43.7±1.8
PPNC3	0.11±0.02	34.9±2.5
PPNC4	0.06±0.01	38.3±3.6
PPNC5	0.15±0.07	29.6±1.7
PPNC6	0.19±0.04	32.5±2.6
PPNC7	0.17±0.03	34.6±3.4
PPNC8	0.24±0.09	27.7±1.8
PPNC9	0.30±0.05	22.1±1.4
PPNC10	0.32±0.07	21.3±2.7
PPNC11	0.26±0.06	26.1±2.6
PPNC12	0.30±0.08	23.6±0.9
PPNC13	0.29±0.02	24.5±0.7
PPNC14	0.33±0.04	22.8±1.4
PPNC15	0.32±0.01	23.3±1.1
BPNC1	0.01±0.006	49.7±3.5
BPNC2	0.01±0.004	51.2±2.4
BPNC3	0.05±0.01	42.6±4.3
BPNC4	0.04±0.008	47.3±3.3
BPNC5	0.11±0.03	40.4±1.6
BPNC6	0.14±0.02	38.9±1.9
BPNC7	0.13±0.06	40.1±2.5
BPNC8	0.18±0.04	35.7±1.7
BPNC9	0.22±0.07	30.5±1.1
BPNC10	0.20±0.05	32.8±2.4
BPNC11	0.18±0.03	35.2±2.9
BPNC12	0.25±0.08	28.5±2.3
BPNC13	0.21±0.06	32.4±2.6
BPNC14	0.28±0.02	26.1±1.8
BPNC15	0.27±0.05	27.3±1.5

*Expressed as Mean±Standard deviation for n = 3, PPNC: Poloxamer Posaconazole Nano-crystals; BPNC: Brij Posaconazole Nano-crystals

Table 3: SMSS analysis results for the responses solubility and T90%

Source	Model p-value	Adjusted R^2	Predicted R^2	Inference
For solubility				
Design Model	<0.0001	0.9357	-0.2623	
Linear	<0.0001	0.9135	0.8932	
2FI	0.8272	0.9007	0.8512	
Quadratic	0.0072	0.9433	0.8932	Suggested
Cubic	0.7888	0.9229	-0.4302	
For T90%				
Design Model	<0.0001	0.9542	0.4058	
Linear	<0.0001	0.9279	0.9100	
2FI	0.2848	0.9334	0.8961	
Quadratic	0.0127	0.9591	0.9220	Suggested
Cubic	0.6174	0.9544	0.3267	

*2FI: 2 Factorial Interaction

Table 4: ANOVA test results of the quadratic models for the responses solubility and T90%

Source	Sum of squares	Degrees of freedom	Mean square	F-value	*p*-value[a]	Inference
For solubility						
Model	2018.08	13	155.24	53.25	<0.0001	Significant
A-Sonication Power	279.46	1	279.46	95.87	<0.0001	Significant
B-Sonication Tiime	104.77	1	104.77	35.94	<0.0001	Significant
C-Stabilizer Concn.	1124.19	1	1124.19	385.64	<0.0001	Significant
D-Stabilizer Type	427.90	1	427.90	146.79	<0.0001	Significant
AB	0.5625	1	0.5625	0.1930	0.6663	
AC	15.60	1	15.60	5.35	0.0343	
AD	0.1354	1	0.1354	0.0464	0.8321	
BC	5.29	1	5.29	1.81	0.1967	
BD	3.94	1	3.94	1.35	0.2619	
CD	12.82	1	12.82	4.40	0.0522	
A^2	1.94	1	1.94	0.6662	0.4264	
B^2	0.7056	1	0.7056	0.2421	0.6294	
C^2	30.41	1	30.41	10.43	0.0052	Significant
Residual	46.64	16	2.92			
Cor Total	2064.72	29				
For T90%						
Model	0.3067	13	0.0236	38.10	<0.0001	Significant
A-Sonication Power	0.0290	1	0.0290	46.84	<0.0001	Significant
B-Sonication Tiime	0.0141	1	0.0141	22.70	0.0002	Significant
C-Stabilizer Concn.	0.2292	1	0.2292	370.09	<0.0001	Significant
D-Stabilizer Type	0.0208	1	0.0208	33.60	<0.0001	Significant
AB	0.0000	1	0.0000	0.0404	0.8433	
AC	0.0004	1	0.0004	0.6460	0.4333	
AD	0.0003	1	0.0003	0.4503	0.5117	
BC	0.0002	1	0.0002	0.3634	0.5551	
BD	0.0005	1	0.0005	0.8195	0.3788	
CD	0.0016	1	0.0016	2.54	0.1305	
A^2	0.0008	1	0.0008	1.35	0.2619	
B^2	0.0004	1	0.0004	0.6277	0.4398	
C^2	0.0082	1	0.0082	13.21	0.0022	Significant
Residual	0.0099	16	0.0006			
Cor total	0.3166	29				

[a]*p*-value<0.05 indicates the model terms are significant

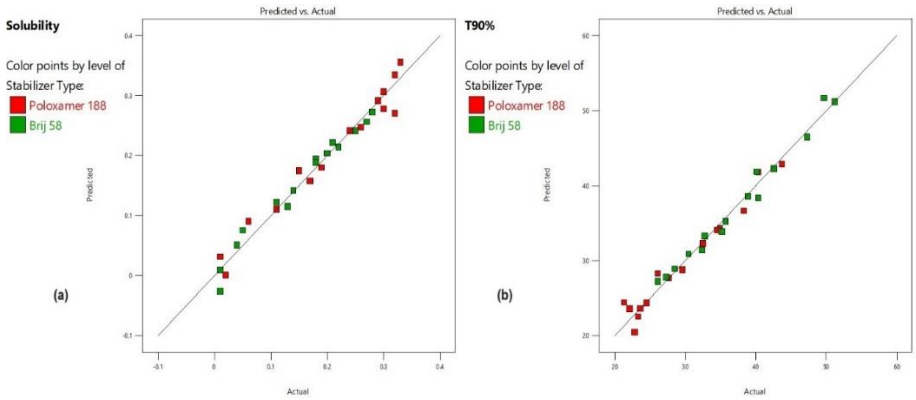

Fig. 1: Predicted vs. Actual plots for the responses (a) Solubility and (b) T90%

DoE analysis of the responses

SMSS analysis results (presented in table 3) showed that the factors had a quadratic effect on the both the responses. For the suggested model, ANOVA was performed (results are shown in table 4) to check the significance of the model as well as the factors' effects on the responses.

The ANOVA test results showed that the quadratic models for the solubility and the T90% were found to be significant at $p<0.05$. Also, all the four factors had significant effects at $p<0.05$ on both the solubility and the T90%. Besides the ANOVA test, Predicted vs. Actual plots for both the responses (fig. 1) illustrated that all the data points were uniformly scattered around the 45 line. This observation indicated that the data can be proceeded for DoE analysis and optimization without the need of transformation [20].

The influences of all the factors on the solubility of PNCs are illustrated in fig. 2 (a) – (d). An increase in the sonication power in terms of the amplitude and sonication time caused the solubility to increase. Sonication at high amplitude produces more energy into the dispersion, which can cause a size reduction of the PNCs to a greater extent. Sonication for a longer time also implies more energy to the dispersion, which may cause a fine size reduction [21]. The PNCs with decreased particle size might result in increased solubility. An increase in the level of stabilizer increased the solubility. Higher concentrations of the surfactants PLX-188 and Brij 54, produce more adsorption of these molecules on the surface of

the PNCs, develop steric barriers, and thus decrease their aggregation and produce smaller particle sizes with increased surface area. The same effect of the non-ionic surfactants was also discussed by Gigliobianco MR *et al.* [22]. Besides, the adsorption of more amounts of these hydrophilic surfactants can make the solid particles' surface more hydrophilic. Similar effects of the surfactants on the surface properties and solubility of the poorly soluble drug Deferasirox were reported by Gulsun T *et al.* [23]. Thus, the PNCs with more surface area and with higher amounts of the adsorbed surfactants resulted in increased solubility of the posaconazole. These obtained results are well correlated with previous reports [24]. The type of stabilizer was also found to influence the solubility of the posaconazole significantly. Among the two stabilizers, PLX-188 increased the solubility of the posaconazole from the PNCs to a greater extent than those prepared by Brij 58. This might be attributed to the higher HLB of the PLX-188, which is 29, than the HLB of the Brij 58, which is 16. PLX-188 with a higher HLB is more hydrophilic and hence improved the solubility of the posaconazole to a greater extent than in the case of Brij 58.

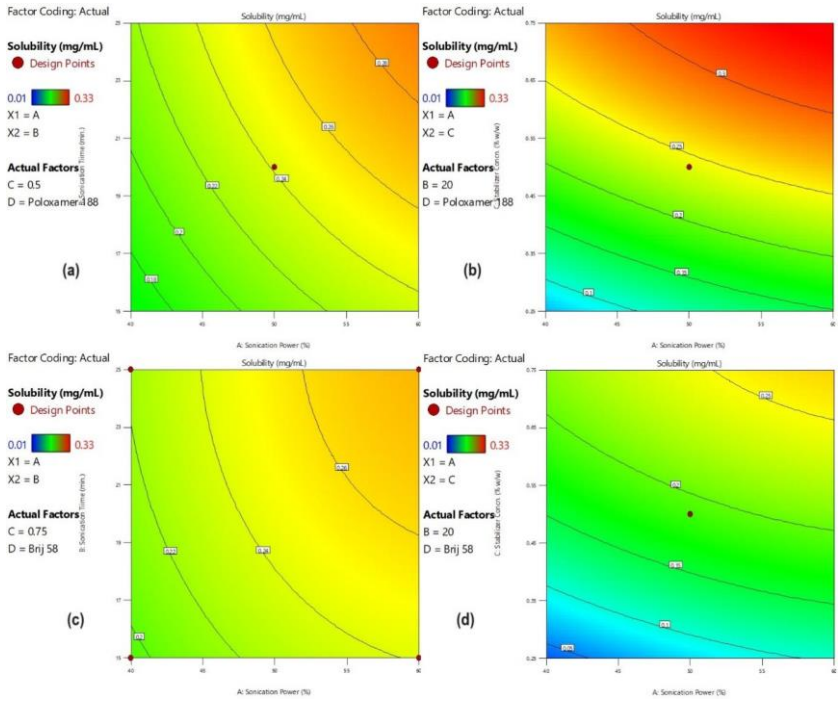

Fig. 2: Displaying influences of the factors (a) A and B, and (b) A and C on the solubility of posaconazole from the PPNCs; influences of the factors (c) A and B, and (d) A and C on the solubility of posaconazole from the BPNCs

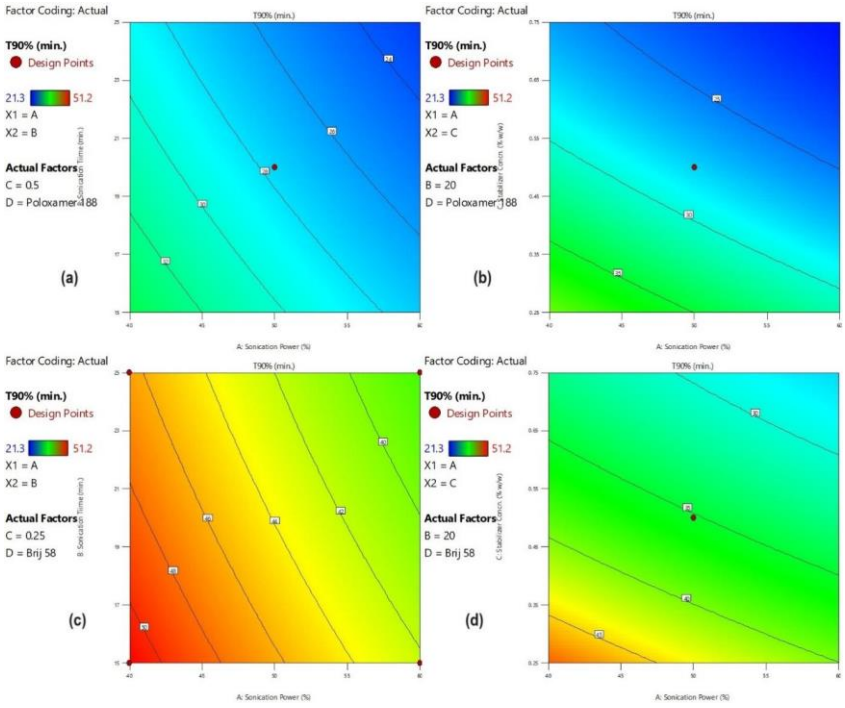

Fig. 3: Displaying influences of the factors (a) A and B, and (b) A and C on the T90% of posaconazole from the PPNCs; influences of the factors (c) A and B, and (d) A and C on the T90% of posaconazole from the BPNCs

The influences of all the factors on the T90% of the PNCs are illustrated in fig. 3 (a) – (d). An increase in the sonication power and sonication time leads to a decrease in T90%, which means an increase in the dissolution rate. These two factors exhibited positive effects on the solubility of the posaconazole, i.e., PNCs prepared at higher sonication amplitude and sonication time exhibited greater solubility of posaconazole. In addition, sonication at higher power could decrease particle size in the dispersion also [25]. This increased solubility and decreased particle size of the posaconazole could cause rapid dissolution from the PNCs, and the T90% was decreased. Upon increasing the level of stabilizer, the T90% of the posaconazole was found to be decreased. Higher concentrations of the surfactants PLX-188 and Brij 54 increased the posaconazole's solubility to a greater extent. Also, surfactants tend to decrease the interfacial tension and promote wetting [26]. These characteristics altogether might result in rapid dissolution with lesser T90%. The type of stabilizer was also found to influence the T90% of the posaconazole significantly. Among the two stabilizers, PLX-188 increased the dissolution rate and decreased the T90% to a greater extent than that of Brij 58. Poloxamers have the ability to minimize the precipitation of drug steric stabilization. This property, along with the higher hydrophilicity of the PLX-188, which could make the PNCs more hydrophilic, might be responsible for the rapid dissolution of posaconazole.

Optimization

The results of optimization were drawn from the software in the form of an overlay plot (shown in fig. 4). In this, the lower limit for maximizing solubility was set at 0.25 mg/ml, i. e., the requirement of solubility above 0.25 mg/ml. And the upper limit for minimizing T90% was set at 30 min, i. e., the requirement of T90% below 30 min. Under these criteria, the obtained overlay plot exhibited a yellow colour region which is called the design space. The design space is the boundary inside which the combination of the factors at any level would yield the product with the desired values of the responses. One such combination of the factors inside the design space was identified by the software with their predicted values of the response, which are shown in table 5.

A new PNCs at these suggested levels of the factors was again prepared and studied for solubility and dissolution rate. The solubility of these PPNCs was found to be 0.29 mg/ml and the T90% was observed to be 24.7 min. These values of the responses were found to be within the range of 95% confidence intervals of the predicted values. Hence, the obtained response values were correlated with those of the predicted values by the software. So, this combination of the PPNCs prepared with 0.75% w/w of PLX-188 by sonicating at an amplitude of 40% for 20 min. was taken as the optimized PNCs.

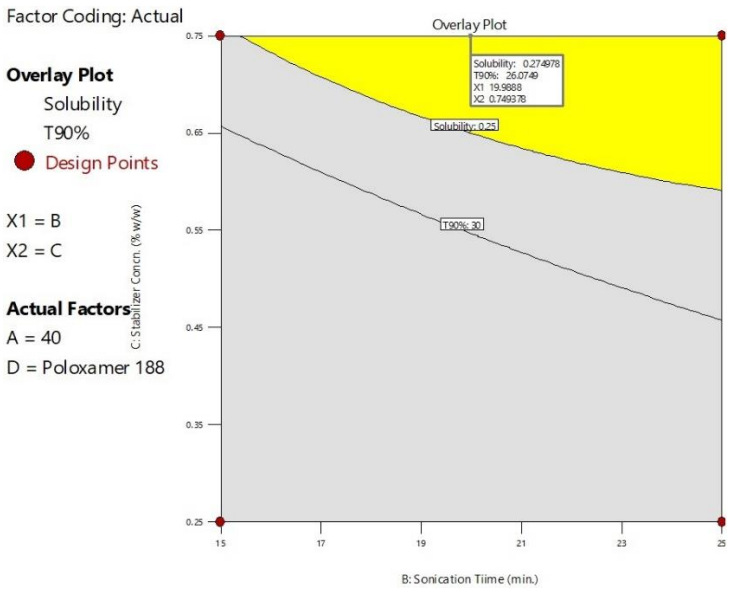

Fig. 4: Overlay plot showing the design space for the set constraints of the PNCs optimization

Table 5: Optimized PNCs combination with the predicted and the observed values of the responses

Factors combination	Responses	Predicted values	95% CI low	95% CI high	Observed values
A: Sonication power. (40% amplitude)	R1: Solubility (mg/ml)	0.28	0.24	0.31	0.29
B: Sonication time (20 min.)	R2: T90% (min.)	26.1	23.9	28.1	24.7
C: Stabilizer concn. (0.75% w/w)					
D: Type of stabilizer (PLX-188)					
*CI: Confidence Intervals; PLX-188: Poloxamer 188					

Particle size and zeta potential

The optimized PNCs were further subjected to particle size and zeta potential studies. The observed results are illustrated in fig. 5 (a) and (b). The particle size was found to be 159.2 nm with a PDI of 0.284, and the zeta potential was found to be-13.8 mV. These characteristics indicate that the PNCs can be easily diffused into the alveolar region without being affected by phagocytosis. But, these nano-sized particles have poor aerodynamic properties and are not suitable for inhalational delivery [27]. Hence, they were subjected to size enhancement without affecting the solubility or dissolution rate for achieving improved aerodynamic properties suitable for inhalation.

Development and characterization of NMPs

The PNCs exhibited significant improvement in solubility as well as dissolution as compared to those of pure posaconazole, which makes posaconazole dissolve readily to produce local action at the site of administration. But, these characteristics alone are not sufficient to administer the PNCs through the inhalational route. Because of the nano-size of the PNCs, their aerodynamic properties are poor and do

not support r inhalational delivery. So, the size of these PNCs should be increased to a micron level below 5 µm so as to have credible aerodynamic properties that are suitable for inhalational delivery. Hence, in this work, the optimized PNCs were embedded into a lactose matrix to produce NMPs. So, these NMPs were studied for several characterization studies to ensure improved aerodynamic properties without affecting their dissolution characteristics.

The prepared NMPs were studied for micromeritic characteristics, including aerodynamic diameter, surface morphology by SEM and DSC studies. Also, the NMPs were subjected to dissolution studies to investigate any changes in the dissolution rate owing to the lyophilization process.

Micromeritic properties of the NMPs

The obtained results of the micromeritic properties are presented in table 6. The mean particle size obtained was 4.42 µm. This was due to the sieving of the NMPs through the 5 µm mesh. This size of the NMPs is highly suitable for delivery through inhalation. Upon inhalation, these size-range particles can reach secondary bronchi, where they can dissolve readily to yield PNCs.

These PNCs can easily escape into alveolar fluids for immediate local action. The aerodynamic diameter and other flow properties indicate that the NMPs can have good nebulization properties that are suitable for inhalation.

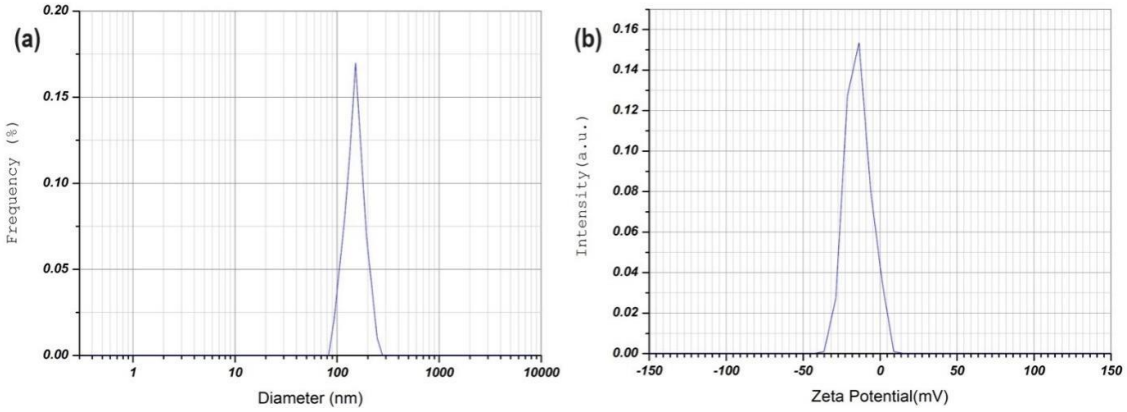

Fig. 5: (a) Particle size distribution and (b) Zeta potential of the optimized PNCs as determined by dynamic light scattering technique using Horiba SZ-100

Table 6: Results of the micromeritic properties of the NMPs

S. No.	Property	Method	Observed results*
1	mean particle size (µm)	Optical microscopy	4.42±0.76
2	True density (g/cc)	Liquid displacement	1.61±0.28
3	Aerodynamic diameter (µm)	Size x (True density)$^{1/2}$	5.61±0.42
4	Bulk density (g/cc)	Bulk density apparatus	0.54±0.03
5	Tapped density (g/cc)	Bulk density apparatus	0.59±0.05
6	Angle of repose ( )	Fixed funnel method	13.62±1.71
7	Carr's index (%)	Bulk density apparatus	8.47±2.69
8	Hausner's ratio	Bulk density apparatus	1.09±0.03

*Expressed as mean±standard deviation for n = 3

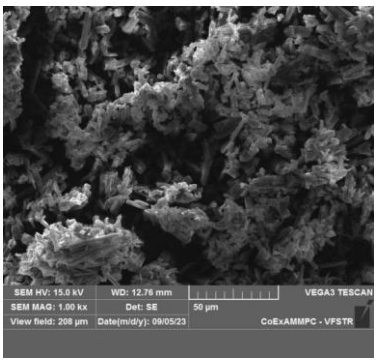

Fig. 6: SEM photographs of the NMPs

SEM studies

The obtained NMPs, after lyophilization followed by sieving, were subjected to SEM studies to understand the surface morphology. The resultant images as shown in fig. 6 illustrated that the NMPs were almost square-shaped with uniform texture. This could be attributed to the lyophilization and sieving process. Even though the NMPs are not exactly spherical, they exhibited good micromeritic properties (table 5) that are necessary for a powder that is to be inhaled in dry form.

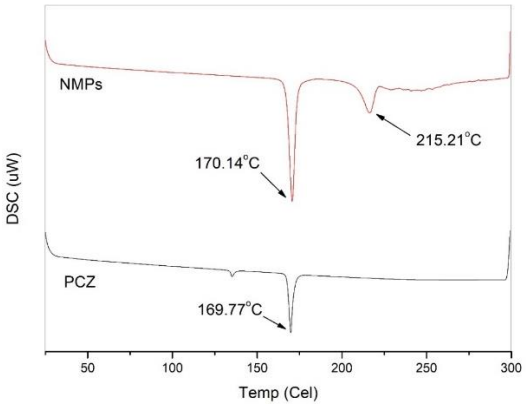

Fig. 7: DSC thermograms of posaconazole nanocrystals and the NMPs

DSC studies

The DSC spectrum of the posaconazole nanocrystals, as shown in fig. 7, exhibited a sharp endothermic peak at 169.77 C. The shape of the peak demonstrated that the drug was in crystalline form. And the peak position corresponds to its melting point temperature. After embedding these nanocrystals in lactose into NMPs, DSC analysis was again conducted. The spectrum of the NMPs demonstrated a sharp endotherm at 170.14 °C.

This observation illustrated that posaconazole was still in its crystalline form, and the embedding process by lyophilization to increase the size did not affect the crystalline nature of the drug. This confirmed that the solubility and dissolution characteristics of the PPNCs would remain the same while adding aerodynamic advantages owing to the increased size upon conversion into NMPs.

Dissolution studies

The dissolution profiles of the pure posaconazole, the optimized PNCs, and the NMPs are shown in fig. 8. These profiles illustrated that the dissolution rate of the posaconazole was greatly increased compared to the pure drug upon preparation of the PPNCs. The T90% of the posaconazole from the pure drug was observed to be 217.4 min. Whereas the T90% of the posaconazole from the optimized PNCs was found to be 24.7 min. It indicated that the dissolution of the posaconazole was increased by 8.77-fold upon the development of the PPNCs. Later, these PPNCs were embedded in the micron-sized lactose matrix to obtain the NMPs. The dissolution profile of the posaconazole from these NMPs was almost similar to that of the PPNCs, with a T90% of 26.1 min. This observation designated that the dissolution of posaconazole remained almost the same and did not change significantly upon conversion of the PPNCs into NMPs. The obtained results were correlated with those reported by Li M *et al.* [28].

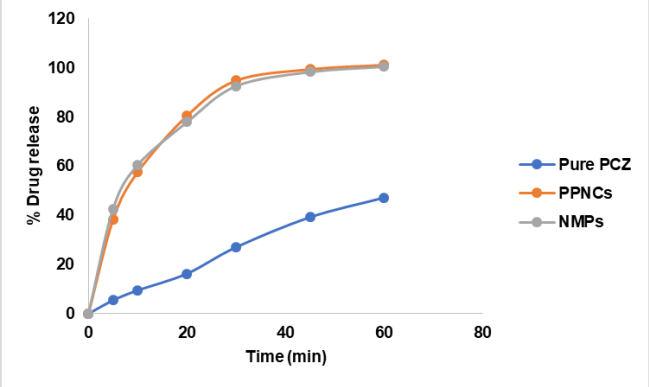

Fig. 8: Comparative dissolution profiles of posaconazole from the pure drug, PPNCs and NMPs. Results are given as mean of triplicate.

CONCLUSION

Nano-crystallization using ultrasonication was employed to prepare PNCs with improved solubility and dissolution rate of the posaconazole. Four different formulation and process variables were studied for their influence on the solubility and dissolution of the posaconazole using CCD. The DoE analysis signified that all the factors had a significant influence on both the solubility and dissolution rate of the posaconazole from the PNCs. The optimization studies to maximize these characteristics produced a formulation of PNCs prepared at 40% amplitude for 20 min. of sonication time with 0.75% w/v of PLX-188 as the stabilizer as the optimized formulation. These optimized PNCs exhibited an 8.8-fold increase in dissolution rate compared to pure posaconazole. Later, these optimized PNCs were embedded into the lactose matrix by lyophilization to yield NMPs. The results of the micromeritic properties and dissolution studies on the NMPs signified that embedding the PNCs into the lactose matrix improved the aerodynamic and micromeritic properties of the NMPs without altering the crystalline and dissolution characteristics of the posaconazole. Henceforth, these NMPs are suitable for inhalational administration into lungs, and thus, the objectives of the work are achieved positively.

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AUTHORS CONTRIBUTIONS

Yallamalli IM contributed in the research idea and work plan. Puvvala S contributed in the execution of the work, obtaining the data and drafting the manuscript. Yallamilli IM contributed in the final review of the manuscript.

CONFLICTS OF INTERESTS

The authors declare no conflicts.

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