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Research Article

Physicochemical and Aerosolization Assessment of Inhalable Spray Dried Fluconazole Powder

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ABSTRACT

Background: Respiratory fungal diseases therapy is still facing challenges as a result of increasing autoimmune disorders, cancers, and immunosuppressive medication usage. Fluconazole is a wide spectrum antifungal agent and is still used successfully in the treatment of opportunistic infections in combination with other antifungal agents. Since, the treatment of respiratory fungal diseases requires prolonged hospitalization; it may increase the chances of other opportunistic infections. Considering the reported drug resistance and adverse effects of systemic administration, it appears that localized pulmonary antifungal therapy may be a suitable alternative route. According to the reported suitable inhalation properties of spray dried powders; spray drying technique was used to prepare fluconazole powders.

Methods: Different spray drying parameters such as inlet temperature, pump rate, aspiration%, solvent type, as well as fluconazole concentration were evaluated for powder production. The optimized formulations were characterized using scanning electron microscopy (SEM), x-ray diffraction (XRD), differential scanning calorimetry (DSC) and aerodynamic parameters.

Results: All selected formulations showed a smooth surface with similar mass median aerodynamic diameter (MMAD) in a respiratory acceptable range. While optimized powder showed a lower geometric standard deviation (GSD) of 1.5 with higher fine particle fraction (FPF) of 26% and almost complete deposition recovery of 97%.

Conclusion: Based on *in vitro* characterization results, it appears that spray drying is an appropriate and cost-effective technique for the production of inhalable fluconazole powder. It is characterized by a narrower size distribution and delivers a higher dose which may be more cost effective for mass production.

Introduction

Despite the less frequent incidence of respiratory fungal diseases, their treatment is still a challenge due to the increase in autoimmune disorders, cancers, and immunosuppressive medication usage.[1] The most common opportunistic fungal infections with a complicated treatment process are *Candidiasis*, *Histoplasmosis*, *Blastomycosis*, and *Cryptococcosis*. Fluconazole, amphotericin B, itraconazole, and posaconazole are the most commonly used agents in the treatment of fungal respiratory diseases.[2,3] Fluconazole is a first-generation antifungal triazole, which has revealed several advantages in comparison to other azoles including a variety of therapeutic effects in almost all *Candidas* and *Cryptococcus neoformans* [4] to some extent. Fluconazole is the most frequent clinical antifungal agent used for the treatment of cryptococcosis.[5,6] The main challenge associated with the high consumption of antifungal agents is drug resistance and it has been reported for different antifungal agents.[2,7]

The advantages of Fluconazole over other antifungal azole agents, include its lower resistance and relatively less common drug interactions.[8] Fluconazole may also be used as an alternative to Amphotericin B in the treatment of *cryptococcal* infection or in the prevention of cryptococcal meningitis recurrence in patients with AIDS. It is also used to prevent fungal infections in patients treated with radiation therapy or cytotoxic medications.[4,9] According to the required prolonged hospitalization for treatment of respiratory fungal diseases and increasing chances of other opportunistic infections and drug resistance, localized therapy seems to be a suitable route of administration.[10] Localized pulmonary delivery is a needle free route of administration with potential

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advantages such as high absorption surface and low by first pass effect compared to other routes.[11] Achieving efficient lung deposition requires the optimization of some critical aerosolization parameters such as MMAD, GSD and FPF.[12] Spray-drying is a suitable and widely used technique for preparing inhalable powders for a variety of drugs. This is because the spray-drying process is easily controllable and may be used to manage particle size, shape, size distributions and surface energy.[13,14] Therefore, non-invasive pulmonary delivery with its remarkable advantages appears to be the best choice for respiratory infection therapies. Thus, the aim of this study was to optimize and characterize Fluconazole spray-dried powders for inhalation therapy.

Materials and Methods

Materials

Fluconazole was purchased from Zaharvi, Iran. Ethanol (EtOH), methanol (MeOH), Tween 80, potassium dihydrogen phosphate, disodium hydrogen phosphate and phosphoric acid were supplied from Merck chemicals (Darmstadt, Germany).

Methods

Fluconazole analysis

A Fluconazole aqueous standard solution was prepared and its absorbance was scanned at 200-400 nm range using spectrophotometer (Ultrospec 2000, Pharmacia Biotech, UK). The maximum wavelength was selected to analysis Fluconazole.

Fluconazole standard curve was plotted in ethanol:water mixture (30:70) as NGI washing solution. Fluconazole powder was dissolved in solvent to make stock solution (500 µg/ml). Serial dilution was used to prepare diluted standard solutions of 250,125, 62.5, 31.25 µg/ml for each solvent. The absorbance of all solutions was determined at the maximum wavelength using spectrophotometer. All concentrations were prepared in three different days. Each concentration was tested triplicate. Calibration curve was validated by linearity, intraday and inter-day precision% and accuracy%.

Intra-day precision was determined by measuring all concentrations under the same experimental conditions on the same day three times. Inter-day precision was calculated from results of samples on three different days. Accuracy was assessed by measuring three standard fluconazole solution (50, 100, and 200 µg/mL) at three different levels (lower, medium, and upper concentration) and comparing the results with theoretical amount.[15]

Powder preparation using spray drying

As shown in Table 1, different spray dry variables (Pump rate, Aspirator%, Inlet temperature and Nozzle) were examined for better powder weight yield%. In the best selected condition (Table 2) different Fluconazole concentrations (0.5, 1, and 2% w/v) in four different solvents (Ethanol, H_2O:Ethanol, MeOH, H_2O:MeOH) were prepared. All formulations were prepared three times (Table 3).

Table 1. Spray dryer preliminary variables.

Parameter	Values
Nozzle (Pa)	7, 8, 9, 10
Aspirator %	60, 70, 80, 90
Inlet Temperature ^{0}C	60, 70, 80, 90
Pump rate (ml/min)	0.5, 1, 1.5

Table 2. Optimized Spray dryer variables.

Parameter	Values
Nozzle (Pa)	10
Aspirator %	90
Inlet Temperature ^{0}C	90
Pump rate (ml/min)	0.5

Morphological evaluations

Particles size and morphology of all selected spray dried powders were observed using scanning electron microscopy (LEO 1430 VP, UK and Germany) after gold sputtered coating.

X-ray diffraction (XRD) analysis

Crystal structures of all selected spray dried powders were determined using X-ray diffraction (D500, SIEMENSE, Germany) operating with Cu Ka X radiation, a voltage of 40 kV, and a current of 30 mA. The scans were conducted at a scanning rate of 2^0/min in the 2Θ range from 5 to 40^0.[12]

Differential scanning calorimetry

Crystallinity, melting point and enthalpy of all selected spray dried powders were studied using differential scanning calorimetry (DSC-60, SHIMADZU, Japan). The device was calibrated using indium. The samples were placed in the aluminum pans and heated under nitrogen at the range of 25 to 250 ° C with the rate of 10^0C/min. The melting point and enthalpy were calculated using the software TA-60WS.[12,16]

Aerodynamic parameter assessment

The aerosol characterization parameters of the all selected spray dried powders were assessed by an aerolizer connected to the next generation impactor (NGI) with pre-separator and USP induction port (Copley Scientific, Nottingham, UK). To assess the delivered drug, the NGI was assembled and operated in accordance with USP General Chapter 601. Hard gelatin capsules (n=3) were filled spray dried powders. To avoid bouncing of deposited particles, all collecting stages were soaked with Tween® 80 ethanolic solution 1% and for complete evaporation of ethanol were placed under the fume hood. A flow meter (DFM 2000, Copley Scientific, Nottingham, UK) was applied to fix flow rate at 60 L/min.[17]

Ethanol: water mixture (30:70) was used for collecting the inhaled powders in all stages and after centrifugation (30 min at 5000 rpm), the amount of Fluconazole in each stage was determined using analysis method. MMAD, GSD, FPF and Deposited Recovery% were calculated using CITAS V3.10 software.[12,17]

Table 3. Optimization factors for fluconazole spray dried powder preparation (4 different solvents and 3 different Fluconazole concentrations)

Solvents	Ethanol: Water		Ethanol		Methanol		Methanol: Water	
Fluconazole amount (g)	F1	0.5	F4	0.5	F7	0.5	F10	0.5
	F2	1	F5	1	F8	1	F11	1
	F3	2	F6	2	F9	2	F12	2

Results

Fluconazole analysis

The maximum wavelength of the fluconazole absorption spectrum was shown at 260 nm. The calibration curves of Fluconazole in ethanol:water mixture (30:70) was plotted at different standard solutions (31.25, 62.5, 125, 250, and 500 µg/ml). The calibration equation and regression (r^2) was Y=0.0021X+0.0016 and r^2=0.9998. Accuracy, inter and intra-day precision of the calibration curve were obtained 98.4±2.1, 97±3.6 and 97.4±1.8 (RSD), respectively.

Powder preparation using spray drying

Spray-dried powders were produced after the final optimization of the spray dryer condition (Table 2). Table 4 shows the results of the weight yield range of the preliminary developed formulations. Considering the product weight yield, the optimum spray drying conditions were selected to prepare F1 to F12 formulations (Table 3) using four different solvents in three different concentrations of Fluconazole solutions. The weight yields (%) of the final formulations are presented in Table 5.

Table 4. Weight yield% of initially developed formulations (n=3).

Solvent	Weight yield (%)
Methanol: Water	5-11
Ethanol	4-5
Methanol	5-7
Ethanol: Water	5-10

Morphological studies

For SEM analysis, raw Fluconazole powder and optimized spray dried powders F3, F6, F9, and F12 were selected. The results were reported in Figure 1.

Table 5. Weight yield% of formulations (n=3).

	Weight yield (%)		Weight yield (%)		Weight yield (%)		Weight yield (%)
F1	10±0.5	F4	10.8±0.9	F7	10.2±1	F10	11.2±0.8
F2	16.1±0.9	F5	16.4±0.7	F8	17.8±0.8	F11	20.2±0.9
F3	19.5±0.3	F6	27.8±0.3	F9	21±0.2	F12	24.6±0.2

Table 6. Melting point (°C) and fusion enthalpy (J/g) of raw fluconazole powder and different formulations.

	Raw fluconazole	F3	F6	F9	F12
Melting Point (°C)	145.88	142.95	142.97	142.94	142.96
Fusion Enthalpy (J/g)	558.31	537.35	387.75	364.73	531.09

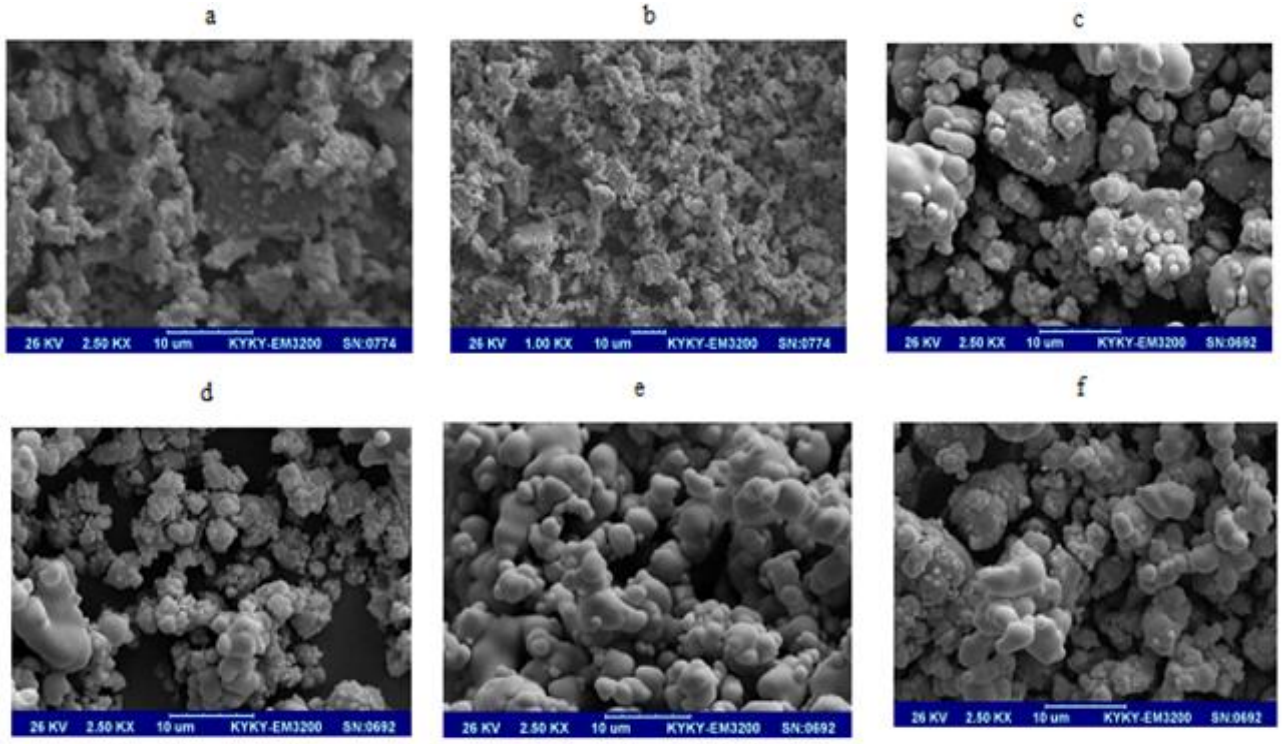

Figure 1. SEM pictures of raw fluconazole powder IKX (a), and raw fluconazole powder (b). F3 (c), F6 (d), F9 € and F12 (f) 2.5KX.

Table 7. Aerosol assessment properties (n=3).

	F3	F6	F9	F12
Deposited recovery%	73.0±2.5	59.9±0.9	96.9±1	78.8± 2.2
FPF (%)	24.8 ± 1.41	17.1± 1.57	25.7± 0.25	20.1 ± 1.92
MMAD(µm)	4.17±0.08	5.00 ±0.19	5.47± 0.06	5.15± 0.28
GSD	2.2±0.03	2.1±0.03	1.5±0.02	2.0±0.06

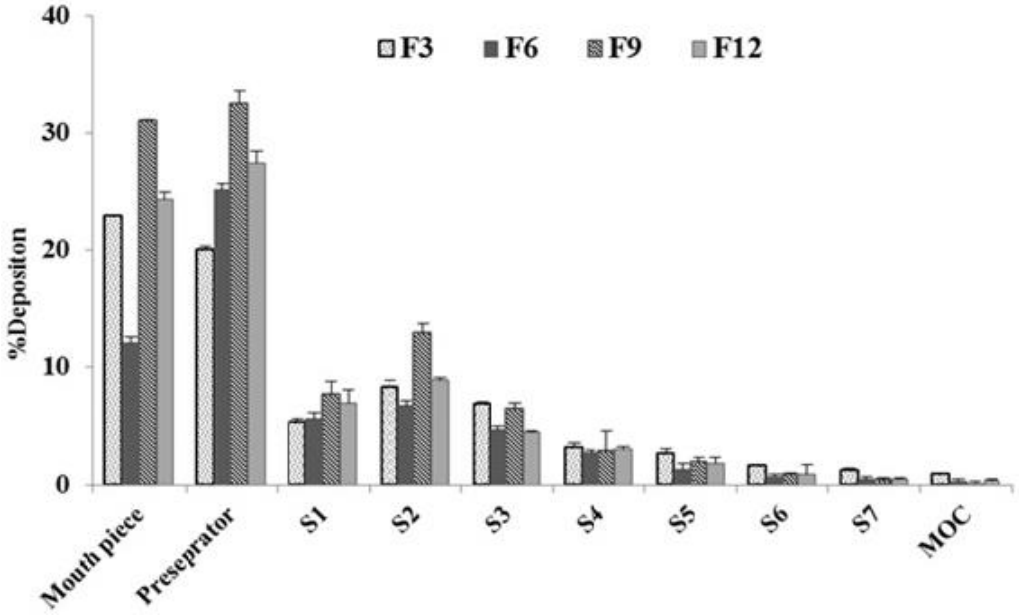

Figure 2. Aerosols in vitro lung deposition diagram (n=3).

XRD analysis

Raw Fluconazole powder and optimized spray dried powders F3, F6, F9, and F12 XRD diffractogram were presented in Figure S1 in Supplementary Materials.

DSC Analysis

Raw Fluconazole powder, F3, F6, F9, and F12 formulations of the DSC thermogram are shown in Figure S2 in Supplementary Materials. The melting point and fusion enthalpy of all formulations are described in Table 6.

Aerodynamic parameter assessment

Figure 2 shows the deposition distribution of all formulations. The inhalation assessment results have been reported in Table 7.

Discussion

For many years, inhaled medications have been approved and widely administered for the treatment of lung diseases as well as an optimal and attractive route of administration.[18] Inhaled drug delivery is one of the most important and interesting routes of administration for local and systemic therapy.[19] Since systemic therapies require higher drug doses which may result to severe adverse effects, the development of inhaled formulations that enable targeted drug delivery to airways with minimal systemic drug exposure is necessary.[20] Compared with other modes of aerosols, dry powder inhalers are solid dosage forms with remarkable advantages such as formulation stability and low cost with a wide variety of design attribution which can be used for performance optimization.[21]

Spray drying is an initial particle engineering technique with scale-up capability which produces appropriate inhalable particles.[20] It is applicable in spray drying to improve the quality and efficiency of the inhaler powder using different procedures such as changing solvent,[22] drug concentration and spray drying conditions.[23,24] Although, spray drying is a great way of generating a dry powder inhaler, designing the parameters to optimize the process for the preparation of powder with specific conditions is still a serious challenge.[25]

The third leading cause of death worldwide and the top leading cause of death in developing countries is lower respiratory tract infections which is a basic global financial burden and results to particular problems. The most susceptible patients to invasive pulmonary fungal infections include those immune-compromised with malignancy, hematologic disease, HIV, cancer and organ transplantation patients. According to the reported high mortality and morbidity rates (40–90%), fungal infections have become a disturbing healthcare problem. Different degrees of systemic toxicities are necessarily associated with traditional and extended treatments of antifungals including Fluconazole which often leads to incomplete treatment and poor therapeutic outcomes. Consequently, the inhalation delivery of anti-fungal agents has been considered as a non-invasive route.[20]

In order to obtain micronized Fluconazole powders with suitable aerodynamic properties, the spray drying method was used. In the best optimized spray drying condition,

three different Fluconazole concentrations (0.5, 1, and 2% w/v) were prepared in four different solvents (Ethanol, Ethanol: water, MeOH, MeOH:Water). The weight yield% of spray dried powders was the first factor for choosing formulations. As earlier predicted, the largest weight yield% with least standard variation was related to the most concentrated formulation of each solvent (2%w/v). Therefore, F3, F6, F9 and F12 were selected and entered in further evaluation experiments.

One of the most important characteristics of particles that could affect the inhaled drug delivery is particle morphology.[26] Based on previous studies, particles with a smooth surface have shown good dispersibility and deposition in the lungs.[27,28] A careful controlling design of the spray drying process can result in particles with spherical shapes.[14] The initial raw Fluconazole powder with non-smooth, coarse particles (larger than 10µm), large aggregates and wide particle size distribution were converted to micronized particles with spherical surface shape and lower particle size (smaller than 10µm) after the spray drying process. It appears that all selected formulations with smooth surfaces may have good inhalation features. Regarding particle size distribution, coarse lumps of raw Fluconazole powder were clearly observed but this problem was eliminated in spray dried formulations. Formulations F3, F6 and F12 showed a wide range of distribution with two different ranges of very large (larger than 10 µm) and very small particles (400nm) while F9 with a narrower distribution as well as less dense and cohesive structure presented better morphology in comparison with others.

Considering the critical role of polymorphism on the bioavailability and safety of medications, it is a very important parameter in pharmaceutical formulations.[29] Fluconazole has been reported to have three different polymorphism forms, consisting of one hydrated form and two anhydrous forms (I, II). Polymorphic anhydrous form I is the most stable one.[29-32] Fluconazole is usually a mixture of anhydrous forms I and II as well as monohydrate.[30,32-34]

Raw fluconazole powder diffractogram in Fig. 2 showed a mixture of fluconazole monohydrate, anhydrate I and anhydrate II forms. The presence of 9.9, 16.3, 25.53, and 29.13 (2Θ) peaks in F3 confirmed the existence of anhydrate I polymorph. Detecting peaks 16.56, 20.92, 22.04, and 5.45 (2Θ) in the F6 diffractogram approved the presence of anhydrate I form. Additionally, the sharp and high intensity peaks at 9.1 and 15.31 (2Θ); also confirmed the presence of high percentages of the monohydrate form in the system. The appearance of 7.87, 15.92, 19.74, and 23.81 (2Θ) peaks with remarkable intensity indicates the partial presence of the polymorphic anhydrate II in the powder structure. Thus, the overall polymorphism state of the F6 formulation was a combination of monohydrate with anhydrate I and II forms. However, as a result of the high intensity of 9.1 and 15.31 peaks, it appears that polymorphic monohydrate appears more strongly than others. In the F9 diffractogram, lower intensity peaks of 16.64, 21.1, 22.13, and 25.52 (2Θ) indicated that lower amounts of powder were in the anhydrate I form. The appearance of sharp peaks at 9.2 and 15.39 (2Θ) with lower intensities showed a lower amount of anhydrous form. The existence of the anhydrate II form was attributed to the 7.95, 15.39, 19.74, and 23.81 peaks. Thus, the F9 formulation represented the higher amounts of anhydrate I and II forms and lower monohydrate form, respectively. In the F12 formulation, the presence of 16.64, 21.3, and 22.53 (2Θ) peaks at higher intensities (especially 16.64) suggested that the amounts of the anhydrate I forms were more compared to other formulations. Conversely, the presence of 2Θ peaks at 9.19 and 15.39, also confirmed that the percentage of the monohydrate form in F12 was relatively the same as the F6 formulation. The presence of peaks 7.94, 15.39, 19.72, and 23.82 also suggests some amounts of anhydrate II forms.

DSC is the most widely used thermal analysis method in pharmaceutical solid state studies.[35] The results of DSC thermograms and fusion enthalpy for selected formulations (F3, F6, F9 and F12) showed the presence of crystalline structures with no significant ($p>0.05$) difference in melting temperature (142.94 -142.97^{0}C) while a 3°C reduction was detected compared to raw Fluconazole powder. The results of melting points in DSC were not considered due to the proximity of all the formulations thermograms; therefore, the differentiating factor in these thermograms was the fusion enthalpy.[36]

Formulations F3 and F12 showed the highest enthalpy, which is very close to the enthalpy of raw Fluconazole powder (a decrease of 4 and 5%) while a remarkable reduction was seen in formulations F6 and F9 (a decrease of 32 and 35%). Higher enthalpies express the presence of metastable crystalline forms in structures.[37]

Considering the higher fusion enthalpy of the anhydrate I polymorphic form compared to the monohydrated form [36] it appears that the amounts of this polymorphic form in formulations F6 and F9 is much less compared to others.

Most aerosoliziation characteristics of an inhalable powder include MMAD, GSD, FPF% and deposited recovery%.[12,38] The most critical parameter for suitable inhalation drug delivery is the aerodynamic diameter. Even needle-shaped particles with high elongation and good MMAD could also be located along the air flow to reach the alveolar region.[39] MMAD is explained as the diameter at which 50% of the particles by mass are larger and 50% are smaller. The suitable aerosol MMAD for peripheral airways and alveolar deposition is between 1–5 µm.[18] The inhalation assessment results in Table 7 indicate an acceptable MMAD for all powders with no significant difference ($p>0.05$). In aerosol studies, a monodisperse aerosol shows a GSD of 1 while a heterodisperse aerosol has a GSD of >1.2.[18] Therefore, the ideal GSD is one but practically, aerosols with a lesser GSD or GSD close to 1.22 are considered as uniform in size.[12] Consequently, it appears that formulation F9 with the least GSD, is the least heterodisperse aerosol compared to others.

FPF is the percentage of particles expected to be deposited

deep within the lungs with an MMAD of 5 µm or less.[17] FPF has been reported for both commercial and research devices in a range of 10–70%, thus the reported FPF for selected formulations (17-26%) seems to be in an acceptable range.[40] It should be noted that the presence of a co-solvent in a spray dry system such as ethanol with lower vapor pressure and volatility may decrease FPF.[41] Also, it has been reported that increasing the inhalation airflow rate in DPIs, typically results to increased FPF. However, higher flows cause higher mouth-throat (MT) region deposition due to turbulence and inertial impaction. The deposited recovery% was defined as the mass of drug delivered from the inhaler (i.e., total amount excluding the inhaler device and capsule).[17,18] According to previous studies, the depositional loss in MT for DPIs was typically in the range of 30–95%.[40,41] Mouth-throat (MT) region deposition for selected formulations was calculated considering the deposited recovery of each formulation which was in the range of 60-66%. It appears that the results were approximately acceptable, since it was reported for improved formulations in a range of 30-40%.[41]

Generally, GSD, FPF and recovery% were the parameters used to select F9 with the most suitable inhalation properties. As it is seen in Fig. 4, F9 showed higher deposition, especially in the S2 and S3 stages which confirm its better aerodynamic properties.

Conclusion

Dry powder inhalers are one of the best formulations for pulmonary drug delivery systems. In this study, different spray-dried powders were prepared and assessed for inhalation characterization. It was found that methanol was the best solvent for the preparation of a suitable respirable powder with acceptable aerosol properties. Optimized formulation showed smooth surface particles with MMAD of 5.47± 0.06 µ and GSD of 1.5 with the FPF of 26% and almost complete deposition recovery (97%). According to *in vitro* characterization, it seems that spray drying was an approximately cost-effective technique for the production of Fluconazole respirable powder with narrower distribution and higher delivered dose which may be more cost effective for mass production.

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Conflict of Interests

The authors claim that there is no conflict of interest.

Supplementary Materials

Supplementary file contains Table S1 and S2 is available on the journal's web site along with the published article.

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