International Journal of Applied Pharmaceutics

ISSN- 0975-7058 Vol 12, Issue 4, 2020

Original Article

POROGEN EFFECTS ON AEROSOLIZATION PROPERTIES OF FLUCONAZOLE LOADED PLGA LARGE POROUS PARTICLES

SHOHREH ALIPOUR[a,b], ATENA SHIROOEE[a], FATEMEH AHMADI[a,c*]

[a]School of Pharmacy, Shiraz University of Medical Sciences, Shiraz, Iran, [b]Pharmaceutical Sciences Research Center, Shiraz University of Medical Sciences, Shiraz, Iran, [c]Research Center for Nanotechnology in Drug Delivery, Shiraz University of Medical Sciences, Shiraz, Iran
Email: Ahmadi_f@sums.ac.ir

Received: 15 Mar 2020, Revised and Accepted: 23 Jun 2020

ABSTRACT

Objective: The most common fungal infection, which is usually occurs in immunocompromised patients, is pulmonary cryptococcosis. Fluconazole (FLZ) is a first-generation triazole which is used for the treatment of pulmonary cryptococcal infection during 6-12 mo. A non-invasive and targeted medication delivery to lung is highly desirable due to lower delivered dose and reduced systemic effects. Large Porous Particles (LPPs) have shown lower phagocytic clearance and higher bioavailability compared to non-porous particles of the same size with a remarkable safety profile.

Methods: In the present study, the effect of two different porogen agents with different mechanisms on FLZ loaded PLGA LPPs properties were evaluated using design expert software®. These properties included volume diameter, drug loading, encapsulation efficiency, mass median aerodynamic diameter (MAAD), geometric standard deviation (GSD) and fine particle fraction (FPF).

Results: All FLZ-loaded PLGA LPPs (FLZ-PLGA LPPs) showed acceptable volume diameter, drug loading and encapsulation efficiency with rapid FLZ release due to macroporous structure. Significant differences in aerosolization properties in which MAAD, GSD and FPF optimized formulation of the optimized formulation were 6.71±0.4 µm, 1.65±0.08 and 33.20±1.7%, respectively.

Conclusion: It was suggested that gas foamed preparation technique using ammonium bicarbonate was a better technique to produce FLZ loaded PLGA LPPs with more suitable *in vitro* respirable properties.

Keywords: Fluconazole, Large porous particle, PLGA, Porogen, Pulmonary delivery

DOI: http://dx.doi.org/10.22159/ijap.2020v12i4.37453. Journal homepage: https://innovareacademics.in/journals/index.php/ijap

INTRODUCTION

Lungs, skin and the central nervous system are the most sensitive organs which are affected by fungal infections very frequently. Pulmonary infection is commonly presented by cryptococcosis, coccidioidomycosis, histoplasmosis, or blastomycosis [1]. The incidence of invasive fungal infection has been increased over the past 30 y following expanded at-risk patients' population, including transplant receivers, patients who have HIV/AIDS or cancer, premature infants and elderly people [2]. The most common fungal infection which is usually occured in immune-compromised patients is pulmonary cryptococcosis, which occurs due to the inhalation of some *Cryptococcus* species' spores. Spores deposition in the alveoli may lead to lung infection [3].

Fluconazole (FLZ) is a first generation triazole with reduced lipophilicity which may be used to prevent and treat mucosal and invasive infections. FLZ is the most common medication for treatment of cryptococcosis and is still used clinically in spite of increasing isolated triazole resistance species. FLZ is recommended to treat acute and chronic pulmonary coccidiodomycosis. The recommended regimen contains oral fluconazole which lasts for 3-6 mo. FLZ is also used for treatment of pulmonary cryptococcal infection for 6-12 mo [4]. Therapeutic agents may deliver to lung via oral, intravenous and pulmonary administration. Oral administration may lead to drug destruction by GI environment or first pass metabolism. Intravenous administration may cause drug accumulation in non-related organs or may lead to drug degradation during circulation. Previous reports indicated lung targeting of azoles would increase local therapeutic effect, minimize systemic exposure and side effects and thus risk of resistances [5]. Therefore, non-invasive and targeted medication delivery to lung is highly desirable due to the lower delivered dose and reduced systemic effects.

Lungs special advantages including large surface area, extensive blood supply and high permeability, make pulmonary delivery a convenient non-invasive route of drug administration [6, 7]. Considering direct delivery of medications into the lung, aerosol therapy may be presented as the most effective treatment tool for lung diseases [8]. Commonly dry powder inhalers mass density is about 1±0.5 g/cm3 with the optimal particle size range of 1–5µm. However, this geometric size is ideal for alveolar macrophage phagocytosis, which reduces aerosols residence time in the lungs [9, 10]. Alveolar macrophages phagocytosis of highly large porous particles (LPPs) with low density (ρ<~0.4 g/cm3) can be significantly diminished and may enhance particle residence time in deep lung [11, 12]. Porous PLGA particles showed remarkable safety profile owing to their compatibility in the human body [13]. In addition, it has been proved that PLGA LPPs could escape macrophage uptake, which leads to efficient delivery of inhaled medicine for long periods of time [14]. Therefore, LPPs have shown better bioavailability compared with non-porous particles of the same size [12, 15, 16].

Poly lactic-co-glycolic acid (PLGA) is a biodegradable, biocompatible and non-immunogenic synthetic polymer which has been approved by FDA for therapeutic targets and is mostly applied among synthetic polymers [17, 18]. To obtain an ideal treatment with lower side effects, it is better to administer high concentrations of therapeutic agents to the target organ directly and continuously [19]. Different typical methods, including gas foaming, porogen leaching and phase separation, are used to generate porous structures in polymer matrices, including microparticles [9]. Porogen leaching method is the most widely used technique with a variety of particulate porogens, such as salts. Effervescent salts, including ammonium bicarbonate are gas-evolving salt porogens which cause carbon dioxide and ammonia gas bubbles vibrant evolution in PLGA structure and produce large porous PLGA scaffolds [20]. Accordingly, pulmonary delivery as an attractive and non-invasive route of administration was chosen for fluconazole LPPs in order to obtain better deposition in the lungs. The objective of this study was to prepare and evaluate inhalable PLGA FLZ-LPPs as a suitable carrier for local delivery into deep lungs using two different pore-forming techniques.

MATERIALS AND METHODS

Chemicals

Fluconazole (FLZ) was received as a gift from Alhavi (Iran), PLGA Resomer® RG502H (lactide: glycolide 50:50, carboxylate end group,

inherent viscosity: 0.18 dl/g) was supplied from Boehringer Ingelheim (Germany), Poly Vinyl Alcohol (MW 72000, 97.5-99.5 mol% hydrolysis) was purchased from Fluka (Sweden). Sodium chloride (NaCl), Ammonium bicarbonate (ABC) and Dichloromethane (DCM) were purchased from Merck (Germany).

Fluconazole analysis

FLZ absorbance was scanned at 200-400 nm range using a spectrophotometer (T80 UV-Vis spectrophotometer (Germany). The maximum wavelength was selected for analysis of Fluconazole.

Fluconazole analysis validation

Two calibration curves were plotted for the determination of FLZ concentration (12.5-200 µg/ml) in two different media (phosphate buffer solution and acetonitrile: water solution). Linearity, inter-day and intra-day precision and accuracy of curves were determined for analytical curve validation [21]. All concentrations were prepared in three different days. Each concentration was tested in triplicate.

Preparation of FLZ-LPPs

FLZ-LPPs were prepared using a modified double emulsion method[20]. PLGA and FLZ (1:1 and 1:2 ratio) were dissolved in 1 ml dichloromethane. Two different porogens were freshly-prepared in their optimized concentration (ABC 1.5% w/v and NaCl 0.5%w/v aqueous solution) and added to the polymer solution. The resulting mixture was sonicated in ice bath for 30s to form a w/o emulsion which was further homogenized in PVA solution (0.5% w/v). The final w/o/w emulsion was added to 5 ml water and stirred overnight at room temperature to remove DCM. The LPPs were collected by centrifugation at 4000 rpm for 15 min, washed 3 times with distilled water and were lyophilized in mannitol 2%.

Considering our previous study, two variable factors were selected for design of the experiment. LPPs were prepared applying 2-level full factorial experimental design using Design expert 10VR software (table 1). Six different responses (volume diameter, drug loading, encapsulation efficiency, Mass median diameter (MMAD), Geometric standard diameter (GSD) and Fine particle fraction (FPF) were examined to obtain the most acceptable formulation for pulmonary delivery.

Solid-state study

Differential Scanning Calorimetry (DSC) was used to study the solid state of prepared LPPs. The thermograms of Blank-LPPs, FLZ and FLZ-LPPs were obtained using DSC BAHR thermoanalyzer (Gmbh, Germany). Samples were weighed and placed in sealed aluminum pans and heating was performed under a flow of nitrogen gas in the range of 25 to 250 °C at a rate of 10 °C/min.

Characterization of FLZ-LPPs

Particle shape and size

Particles shape was analyzed using a light microscope. Mean volume diameter of the FLZ-LPPs was measured by Shimadzu particle size analyzer (SALD 2101, Japan).

Drug loading and encapsulation efficiency

To determine drug loading and encapsulation efficiency, 5 mg FLZ-LPPs were dissolved in DCM and FLZ was extracted using a mixture of water and acetonitrile. Solvents were evaporated under nitrogen flow [10, 22]. FLZ was determined using a validated analysis method. All assays were done in triplicate. Drug loading and encapsulation efficiency were calculated using equations which were mentioned previously.

***In vitro* release profile**

5 mg of FLZ-LPPs were suspended in a tube containing 10 ml phosphate buffer solution (pH, 7.4). Samples were shaken vertically at 100 rpm, 37 °C. At determined time intervals, samples were withdrawn, diluted with acetonitrile and water mixture and centrifuged at 15000 rpm for 10 min. FLZ amount in the supernatant was determined using validated analysis method. The release studies were done in triplicate.

***In vitro* inhalation properties**

FLZ-LPPs powder aerosolization properties were analyzed at room temperature using a 7-stage NGI cascade impact or (Copley Scientific, UK) connected to a Copley HCPS pump, while the airflow rate was set at 30 L/min. 10 mg FLZ-LPPs was delivered into the NGI using a spin haler for every run. Every sample was tested in triplicate. Drug solution was recovered from each collection cup and the amount of the active ingredient in each cup was determined using validated analysis method. Copley Inhaler Testing Data Analysis Software (CITDAS) was used to determine MMAD, GSD and FPF based on drug collected on stages 1–7 and micro-orifice collector (MOC) [23].

Statistical analysis

One-way ANOVA statistical test was used to assess the significance of the differences between the various groups. Multiple comparison Tukey test was used to compare the means of different treatment groups and $P<0.05$ was considered to be statistically significant.

RESULTS AND DISCUSSION

Fluconazole analysis

The maximum wavelength selected for analysis of FLZ was 260 nm.

Analysis method validation

Two different calibration curves were plotted and validated for *in vitro* release (phosphate buffer media), and determination of loading, encapsulation efficiency and aerosolization properties (acetonitrile/water media). The validation parameters including linearity (regression equation, correlation coefficient), inter-day, intra-day precision and accuracy were reported in table 1 which represented a linear standard calibration curve with acceptable accuracy and precision in both experimented media.

Preparation of FLZ-LPPs

Production of controlled size porous particles using porogens has been challenging in recent years. The commonly used technique for the production of PLGA-based particles is double emulsion-solvent evaporation technique which forms an internal and external osmotic pressure difference in the aqueous phases of the emulsion [24]. Polymer/solvent mixture in which porogen salts is embedded forms pores while salts are leached out after solvent evaporation [20]. In particle hardening step, osmogens presence in the internal aqueous phase may cause water influx from the external aqueous phase to internal phase during solvent evaporation, which will form porous particles [24]. Sodium chloride (NaCl) has been used as the osmogen previously. Different concentrations of NaCl in the internal and external aqueous phases may cause different osmotic pressure on the produced microparticles [13]. Previous reports indicated that NaCl was practically an unsuitable agent for the production of PLGA LPPs using double emulsion solvent evaporation technique mainly because of its negative effect on primary emulsion stability [24]. Effervescent based (gas-foamed) techniques are alternative strategies for PLGA LPPs production. In these techniques, an effervescent material such as ammonium bicarbonate is decomposed into carbon dioxide and ammonia during emulsification and can form a porous matrix as the carbon dioxide gas escapes[25]. Two variable factors, porogen type and FLZ amount were selected for different formulations by. FLZ loaded PLGA LPPs were prepared applying a double emulsion solvent evaporation method. A 2-level full factorial experimental design (Design expert®) was used to evaluated six responses, which were explained in table 2.

Table 1: Analytical curves validation results

Solvent	Equation	r^2	Precision% (Intraday)	Precision% (Interday)	Accuracy%
Phosphate buffer	y=0.0033x-0.0076	0.9997	97.4±3.3	91.3±4.3	97.7±2.3
Water/Acetonitril	y=0.0019x-0.0112	0.9994	98.6±1.4	98.3±2.3	94.7±3.2

Table 2: Factors, factor levels and responses in 2-level full factorial experimental design

Factors	Type of factors	Factors level		Response	
X1	Porogen type	ABC	NaCl	Y1	Drug loading%
X2	Fluconazole (mg)	10	20	Y2	Encapsulation efficiency%
				Y3	Volume diameter (micron)
				Y4	MMAD (micron)
				Y5	GSD
				Y6	FPF%

Solid-state study

As it can be seen in fig. 1, FLZ showed an endothermic melting point at 147.5 ^{0}C (C). Blank PLGA LPPs showed an endothermic peak at 162.6 ^{0}C (a). FLZ-LPPs showed both endothermic peaks at 152.3 and 163.9 ^{0}C. Blank PLGA LPPs showed an endothermic peak at 162.6 ^{0}C which is seen in FLZ-LLPs thermogram with a little increase in melting point at 163.9 ^{0}C. DSC thermograms in fig. 5 indicated that the free FLZ endothermic peak at 147.5 ^{0}C is seen in FLZ-LPPs with a little increase in melting point at 152.3 ^{0}C. Considering no new peak appearance or existing peak disappearance, it was confirmed that FLZ was intact during the preparation process and its structure was not destroyed and was incorporated into PLGA LPPs intact.

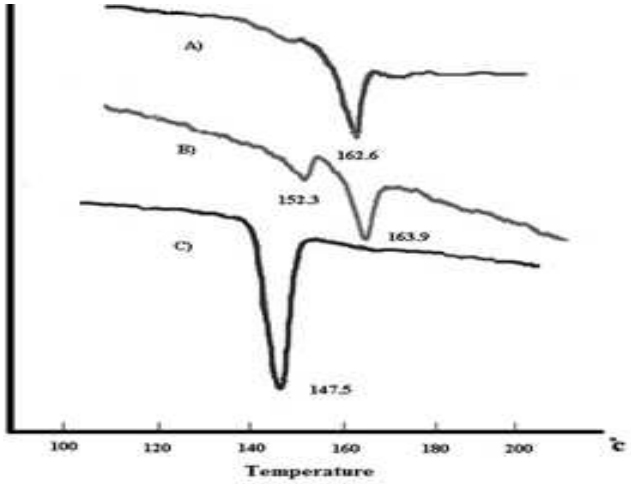

Fig. 1: DSC thermograms of blank-LPPs (a), FLZ-LPPs (b), FLZ (c)

Characterization of FLZ-LPPs

Particle shape and size

Microscopic image of non-freeze dried FLZ-LPPs is seen in fig. 2 in two different scales. As routine non-freeze dried FLZ-LPPs size was less than freeze-dried FLZ-LPPs. Microscopic images could support particle size data obtained from particle size analyzer. Volume diameter (dv) of FLZ-LPPs was in the range of 11-16 µm (table 3). Volume diameter (dv) of FLZ-LPPs was in the range of 11-16 µm. As it is comparable with reported particle size in table 3, FLZ-LPPs particle size in F2 was smaller than F4 but showed higher polydispersity. Particles porosity has been reported by SEM in previous publications [13]. Considering Deign expert software, there was no significant difference ($p>0.05$) in volume diameters of FLZ-LPPs and all particles were in an acceptable range for pulmonary delivery. Results were in consistency with previous reports on PLGA LPPs [24].

Fig. 2: Microscopic images of non-freeze dried FLZ-LPPs F2 (left), F4 (right), Bar= 10000 nm

Drug loading and encapsulation efficiency

Drug loading and encapsulation efficiency for FLZ-LPPs were reported in table 3. Statistical analysis showed that there was no significant difference between drug loading and encapsulation efficiency of F1 to F4 formulation. Drug loading and encapsulation efficiency of F1 to F4 formulation were the same. No significant difference was reported between them. Poor drug encapsulation

efficiency control owing to drug loss between the two phases during particle hardening is the major limitation of osmogen-based technological approach. Previous studies reported higher drug encapsulation efficiency for highly porous PLGA particles since gas-foamed techniques pore formation depends on effervescence rather than diffusional mass exchanges between aqueous phases [24, 25] Hereby, our results indicated that there was no significant difference in drug loading and encapsulation efficiency of all formulations ($p>0.05$) and both groups showed low drug loading efficiency which may be related to large surface area of highly porous particles [9].

Table 3: FLZ-LPPs characterization results

	Porogen type	Fluconazole (mg)	D_v	DL%	EE%
F1	NaCl	10	16.2±2	11.6±0.15	35±0.45
F2	NaCl	20	13.80±1.06	12.2±0.34	24±0.68
F3	ABC	10	15.81±0.82	11±0.51	33±1.53
F4	ABC	20	11.5±0.32	15.3±0.87	31±1.73

D_v: Volume diameter, DL%: Drug loading%, EE%: Encapsulation efficiency%

In vitro release profile

Fig. 3 compares *in vitro* release profiles of different FLZ-LPPs. *In vitro* release profile showed that FLZ was released completely from F2 and F4 formulations after 4 and 6 h while F1 and F3 released FLZ within 8 h. *In vitro* release profiles of different FLZ-LPPs showed a burst release within the first hour was predictable for all PLGA FLZ-LPPs, since porous microparticles may release drugs faster than their solid equivalents, in the same size (Rivera, Martinez-Oharriz *et al.* 2004). Polymer nature may affect FLZ release from FLZ-LPPs. Therefore, the FLZ fast release is related to higher hydrophilicity of PLGA 502H comparing to other PLGA polymers [26]. Drug release in porous microparticles shows faster rate than their solid equivalent in given particle size, considering lower resistance to drug diffusion in porous microparticles [27]. Hence it is obviously predictable that *in vitro* drug release profile of PLGA LPPs would be faster owing to their larger surface area [9]. As it can be seen in fig. 3, higher burst release of formulations F2 and F4 may be related to higher surface adsorbed fluconazole due to the presence of higher initial fluconazole amounts [28, 29]. A rapid drug release due to the macroporous structure of the system is the main drawback of osmogen-based approach [24, 25]; therefore, it can explain the reason of faster release in F1 versus F3.

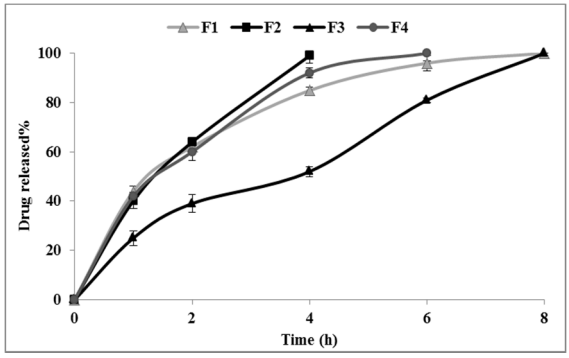

Fig. 3: *In vitro* release profile of FLZ-LPPs. Data represents mean±SD, (n=3)

Possible release kinetics of FLZ-LPPs were evaluated and reported in table 4. Considering higher r^2, Higuchi was the best-fitted model for FLZ-LPPs. Considering higher r^2, the Higuchi model was the best-fitted model for all FLZ-LPPs.

Table 4: Release kinetic R-square results (n=3)

	Zero	First	Higuchi
F1	0.8875	0.9537	0.9999
F2	0.9586	0.9566	0.9999
F3	0.9559	0.905	0.9856
F4	0.9386	0.9811	0.9962

In vitro inhalation properties

Aerosol critical parameters including MMAD, GSD and FPF of FLZ-LPPs were summarized in table 5. MMAD of all formulations were in an acceptable range, while GSD showed upper limit amounts except for F4.

Fig. 4 shows the *in vitro* lung distribution of FLZ-LPPs. Considering design expert® software, three responses (MMAD, GSD and FPF) of FLZ-LPPs were significantly different ($p<0.05$) in F1-F4 formulations. Critical aerosol parameters, including MMAD, FPF and GSD represent particles aerosolization efficacy [30]. The optimum aerodynamic diameter for aerosols is 1–5 µm. Slow settling in smaller particles leads them to be exhaled, while larger particles deposit in the oral cavity or upper airways, which causes their simple clearance [30]. FPF represents the respirable aerosols which are able to deposit in pulmonary tract. Consequently, the deeper lung deposition requires the higher FPF [31]. The ratio of particles with diameter at the 84.1% cumulative percentage to the 50% is defined as GSD and its acceptable range is 1.3–3.0 [32]. Aerosolization properties of all FLZ-LPPs were determined and reported in fig. 5. F1 and F4 showed MMAD a little more than acceptable range while F2 and F3 were in the range. GSD of all formulations was out of range except for F4. The FPF was in the range of 33-74%. Results indicated that for both porogens, FLZ amount significantly affected MMAD of FLZ-LPPs but in a reverse mode. For NaCl, higher fluconazole FLZ amount and for ABC lower FLZ amount produced FLZ-LPPs with smaller and more acceptable MMAD. For GSD and FPF this is quite opposite which means for NaCl, lower FLZ amount and for ABC higher FLZ amount produced FLZ-LPPs with smaller and more acceptable GSD. Considering previous reports, ammonium bicarbote is a better porogen comparing NaCl, since NaCl may form a higher viscous solution due to the interaction of inorganic salts with PVA which leads to form aggregates and gel by salting out [33, 34].

Table 5: FLZ-LPPs aerosolization properties

	MMAD	GSD	FPF%
F1	6.49±0.37	3.19±0.21	36.42±0.53
F2	2.91±1.37	6.62±0.79	74.01±3.09
F3	2.62±0.04	7.71±0.17	51.66±4.73
F4	6.71±0.39	1.65±0.08	33.20±1.69

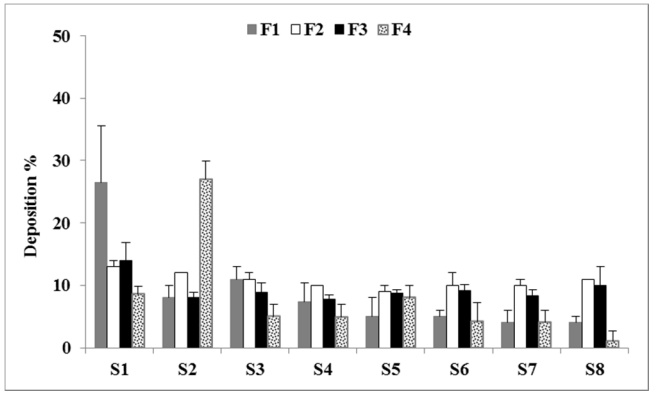

Fig. 4: Aerosol assessment profile of FLZ-LPPs (n=3)

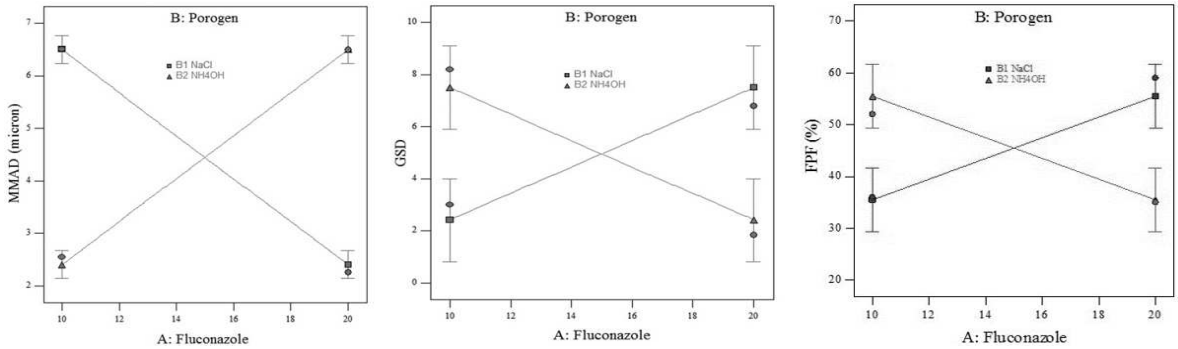

Fig. 5: Interaction plot of MMAD (left), GSD (middle) FPF(right) as dependent parameters (n=3)

Interaction plots and statistical analysis results of design expert®software for aerosolization properties, including MMAD, GSD and FPF were presented in fig. 5 and table 6.

Considering software, "Adeq Precision" measures the signal to noise ratio. A ratio greater than 4 is desirable, which indicates that the model can be used to navigate the design space. All three responses ratio (MMAD, GSD and FPF) indicate adequate signals. The "Pred R-Squared" less than 0.2 is in reasonable agreement with the "Adj R-Squared". The coded equation is useful for identifying the relative impact of the factors by comparing the factor coefficients. As it is shown in equations, MMAD, GSD and FPF were dependent to both factors; porogen type and fluconazole amount.

Table 6: FLZ-LPPs significant responses analysis of variance (A: Fluconazole amount, B: porogen type)

	MMDA	GSD	FPF
p-value prob>F	0.0014	0.0304	0.0299
R-Squared	0.9972	0.9401	0.9412
Adj R-Squared	0.9959	0.9102	0.9118
Pred R-Squared	0.9890	0.7605	0.7647
Adeq Precision	38	7.925	8
Equation	4.7+1.9*AB	4.63-2.17*AB	45.5-10*AB

CONCLUSION

FLZ-PLGA LPPs were prepared using a simple and efficient w/o/w emulsion containing an effervescent porogen. The optimized FLZ-PLGA LPPs (F4) showed suitable aerosolization properties, with Higuchi matrix controlled diffusion release kinetics of fluconazole. Aerosolization properties of F4 were suitable and may confirm *in vivo* efficacy of FLZ-PLGA LPPs in further studies. It confirms the higher efficiency of ABC as the porogen agent for PLGA LPPs.

FUNDING

Nil

AUTHORS CONTRIBUTIONS

Authors declare that the work done by the names mentioned in the article and all the liabilities and claims related to the content of the article will be borne by the authors.

CONFLICT OF INTERESTS

The authors declare that no conflict of interest associated with this work.

REFERENCES

1. Rieder Nelissen CM, J Hasse, RA Yeates, E Sarnow. Fluconazole concentrations in pulmonary tissue and pericardial fluid. Infect Immun 1997;25:192-4.
2. Naggie S, JR Perfect. Molds: hyalohyphomycosis, phaeohyphomycosis, and zygomycosis. Clin Chest Med 2009;30:337-53.
3. Li L, L Zhuang, J Zhou, C Shao. Pulmonary cryptococcosis coexisting with adenocarcinoma: a case report and review of the literature. J Med Case Reports 2018;12:327.
4. Ribas E Ribas AD, P Spolti, EM Del Ponte, KZ Donato, H Schrekker, AM Fuentefria. Is the emergence of fungal resistance

to medical triazoles related to their use in the agroecosystems? A mini-review. Braz J Microbiol 2016;47:793-9.

5. Garbuzenko OB, M Saad, S Betigeri, M Zhang, AA Vetcher, VA Soldatenkov, *et al.* Intratracheal versus intravenous liposomal delivery of siRNA, antisense oligonucleotides and anticancer drug. Pharm Res 2008;26:382-94.
6. Alipour S, H Montaseri, A Khalili, M Tafaghodi. Non-invasive endotracheal delivery of paclitaxel-loaded alginate microparticles. J Chemother 2016;28:411-6.
7. Arafa M, B Ayoub. Nano-vesicles of salbutamol sulphate in metered-dose inhalers: formulation, characterization and *in vitro* evaluation. Int J Appl Pharm 2017;9:100-5.
8. Hu Y, M Li, M Zhang, Y Jin. Inhalation treatment of idiopathic pulmonary fibrosis with curcumin large porous microparticles. Int J Pharm 2018;551:212-22.
9. Lee J, YJ Oh, SK Lee, KY Lee. Facile control of porous structures of polymer microspheres using an osmotic agent for pulmonary delivery. J Controlled Release 2010;146:61-7.
10. Yang Y, N Bajaj, P Xu, K Ohn, MD Tsifansky, Y Yeo. Development of highly porous large PLGA microparticles for pulmonary drug delivery. Biomaterials 2009;30:1947-53.
11. Edwards DA, J Hanes, G Caponetti, J Hrkach, A Ben Jebria, ML Eskew, *et al.* Large porous particles for pulmonary drug delivery. Science 1997;276:1688-71.
12. Oh YJ, J Lee, JY Seo, T Rhim, SH Kim, HJ Yoon, *et al.* Preparation of budesonide-loaded porous PLGA microparticles and their therapeutic efficacy in a murine asthma model. J Controlled Release 2011;150:56-62.
13. Nasr M, GAS Awad, S Mansour, I Taha, AA Shamy, ND Mortada. Different modalities of NaCl osmogen in biodegradable microspheres for bone deposition of risedronate sodium by alveolar targeting. Eur J Pharm Biopharm 2011;79:601-11.
14. Ungaro F, G De Rosa, A Miro, F Quaglia, MI La Rotonda. Cyclodextrins in the production of large porous particles: development of dry powders for the sustained release of insulin to the lungs. Eur J Pharm Sci 2006;28:423-32.
15. Kim I, HJ Byeon, TH Kim, ES Lee, KT Oh, BS Shin, *et al.* Doxorubicin-loaded highly porous large PLGA microparticles as a sustained-release inhalation system for the treatment of metastatic lung cancer. Biomaterials 2012;33:5574-83.
16. Chow A, H Tong, P Chattopadhyay, B Shekunov. Particle engineering for pulmonary drug delivery. Pharm Res 2007;24:411-37.
17. Rajendran R, R Balan, N Ganesan, D Thiruvengadam. Recent modalities in drug delivery via inhalation therapy–an advanced treatment strategy for pulmonary carcinoma. Int J Pharm Pharm Sci 2015;7:8-21.
18. Vakilinezhad MA, A Amini, T Dara, S Alipour. Methotrexate and curcumin co-encapsulated PLGA nanoparticles as a potential breast cancer therapeutic system: *in vitro* and *in vivo* evaluation. Colloids Surfaces B 2019;184:1105-15.
19. Koshkina NV, JC Waldrep, LE Roberts, E Golunski, S Melton, V Knight. Paclitaxel liposome aerosol treatment induces inhibition of pulmonary metastases in murine renal carcinoma model. Clin Cancer Res 2001;7:3258-62.
20. Kim TK, JJ Yoon, DS Lee, TG Park. Gas foamed open porous biodegradable polymeric microspheres. Biomaterials 2006;27:152-9.
21. Parhizkar E, L Emadi, S Alipour. Development and evaluation of midazolam in situ nasal gel properties in presence of solubility enhancers at cilia-friendly pH. Macromol Res 2017;25:225-61.
22. Seema Kohli, Abhisek Pal, S Jain. Preparation, characterization and evaluation of poly (lactide-co-glycolide) microspheres for the controlled release of zidovudin. Int J Pharm Pharm Sci 2017;9:70-7.
23. Doan TVP, JC Olivier. Preparation of rifampicin-loaded PLGA microspheres for lung delivery as an aerosol by premix membrane homogenization. Int J Pharm 2009;382:61-6.
24. Ungaro F, C Giovino, C Coletta, R Sorrentino, A Miro, F Quaglia. Engineering gas-foamed large porous particles for efficient local delivery of macromolecules to the lung. Eur J Pharm Sci 2010;41:60-70.
25. Ungaro F, I d'Angelo, A Miro, MI La Rotonda, F Quaglia. Engineered PLGA nano-and micro-carriers for pulmonary delivery: challenges and promises. J Pharm Pharmacol 2012;64:1217-35.
26. Rivera PA, MC Martinez Oharriz, M Rubio, JM Irache, S Espuelas. Fluconazole encapsulation in PLGA microspheres by spray-drying. J Microencapsul 2004;21:203-11.
27. Arnold MM, EM Gorman, LJ Schieber, EJ Munson, C Berkland. Nano cipro encapsulation in monodisperse large porous PLGA microparticles. J Controlled Release 2007;121:100-9.
28. Ansary RH, MB Awang, MM Rahman. Biodegradable poly(D,L-lactic-co-glycolic acid)-based micro/nanoparticles for sustained release of protein drugs-a review. Trop J Pharm Res 2014;13:1179-90.
29. Makadia HK, SJ Siegel. Poly lactic-co-glycolic acid (PLGA) as biodegradable controlled drug delivery carrier. Polymers 2011;3:1377-97.
30. El-Gendy N, C Berkland. Combination chemotherapeutic dry powder aerosols via controlled nanoparticle agglomeration. Pharm Res 2009;26:1752-63.
31. Naikwade S, A Bajaj, P Gurav, M Gatne, P Singh Soni. Development of budesonide microparticles using spray-drying technology for pulmonary administration: design, characterization, *in vitro* evaluation and *in vivo* efficacy study. AAPS PharmSciTech 2009;10:993-1012.
32. Liu XB, JX Ye, LH Quan, CY Liu, XL Deng, M Yang, *et al.* Pulmonary delivery of scutellarin solution and mucoadhesive particles in rats. Eur J Pharm Biopharm 2008;70:845-52.
33. Lin HR, CJ Kuo, CY Yang, SY Shaw, YJ Wu. Preparation of macroporous biodegradable PLGA scaffolds for cell attachment with the use of mixed salts as porogen additives. J Biomed Mater Res 2002;63:271-9.
34. Zheng WW, L Zhao, YM Wei, Y Ye, SH Xiao. Preparation and the *in vitro* Evaluation of the nanoemulsion system for the transdermal delivery of granisetron hydrochloride. Chem Pharm Bull 2010;58:1015-9.