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Research Article

Initial Characterization of Micafungin Pulmonary Delivery *via* Two Different Nebulizers and Multivariate Data Analysis of Aerosol Mass Distribution Profiles

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Abstract. Pharmaceutical aerosols have been targeted to the lungs for the treatment of asthma and pulmonary infectious diseases successfully. Micafungin (Astellas Pharma US, Deerfield, IL, USA) has been shown to be an effective antifungal agent when administrated intravenously. Pulmonary delivery of micafungin has not previously been reported. In the present pilot study, we characterize the performance of two nebulizers and their potential for delivering micafungin to the lungs as well as the use of multivariate data analysis for mass distribution profile comparison. The concentration of micafungin sodium increased by 21% when delivered by the Acorn II nebulizer and by 20% when delivered by the LC Plus nebulizer, respectively, from the first to the second sampling period. The Acorn II nebulizer delivered a fine particle fraction $FPF_{5.8}$ (%<5.8 μm) of 92.5±0.8 and $FPF_{3.3}$ (%<3.3 μm) of 82.3±2.1 during the first sampling period. For the LC Plus nebulizer, $FPF_{5.8}$ was 92.3±0.1 and $FPF_{3.3}$ was 67.0±0.7 during the first sampling period. The mass median aerodynamic diameter (MMAD) increased from 1.67±0.05 to 1.77±0.04 μm (Acorn II nebulizer) and from 2.09±0.01 to 2.20±0.01 μm (Pari LC Plus nebulizer) from the first to the second sampling periods. These changes in MMAD were statistically significant by paired *t* test. Multivariate data analysis showed that this could be explained systematically by greater drug deposition on stages with larger cutoff sizes and reduced drug deposition on stages with smaller cutoff sizes rather than multimodal deposition or other anomalies in size distribution.

KEY WORDS: impactor profile comparison; micafungin; multivariate data analysis; nebulizer.

INTRODUCTION

Micafungin sodium is an echinocandin antifungal agent (1) that was approved by the United States Food and Drug Administration in March 2005. The drug has been proven effective for prophylaxis against candidal infections in patients undergoing hematopoietic stem cell transplantation and for the treatment of esophageal candidiasis and invasive candidiasis (2). Micafungin was synthesized from a natural product of the fungus *Coleophama empedri* by certain modifications to improve its potency (3). Micafungin acts as a noncompetitive inhibitor of the enzyme, 1,3-β-D-glucan synthase, an enzyme unique to fungi which is necessary for synthesis of 1,3-β-D-glucan. 1,3-β-D-Glucan is essential for osmotic stability and integrity of the cell wall of several common fungal pathogens (4,5). Micafungin exhibits fungistatic activity against *Aspergillus* spp. but is fungicidal against *Candida* spp., which may be explained by the difference in the relative abundance of 1,3-β-D-glucan in the fungal cell wall of the fungi (6–8). Due to large molecular weight and poor oral bioavailability, micafungin can only be administered intravenously. Pharmacokinetic studies of micafungin suggest a linear dose-dependent relationship (9) in both pediatric and adult patients with a half-life of 14.6±3 h (10). Micafungin is widely distributed into various tissue including liver, kidney, and lung tissues as demonstrated in rat and rabbit models (11,12). The drug utilizes biliary excretion as its primary route of elimination and is, therefore, not a concern when used in patients with renal dysfunction (2).

Aerosol delivery of drugs has been widely adopted especially in asthma therapy and has been studied for many agents including corticosteroids, bronchodilators, antibiotics, and antifungal agents (13,14). Delivery devices include propellant-driven metered dose inhalers (pMDIs), dry powder inhalers (DPIs), and nebulizers (15). Nebulizers are used to administer drugs in the form of a liquid mist to the airways. Large quantities of drug can be administrated in this manner compared to pMDIs and DPIs. In contrast to pMDIs and DPIs which administrate bolus doses of drugs, nebulizers deliver drugs in a continuous manner. Aerosol delivery of drugs can achieve greater local concentrations in the lung, which is frequently the site by which many pathogens gain entry into the body. The wisdom regarding aerosol delivery of antimicrobials lies in the potential to deliver high concentrations of drug that exceed the inhibitory concentrations for target pathogens directly to the site of initial colonization and subsequent infection, the lung. The quantity of drug delivered may exceed

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attainable pulmonary concentrations when the drug is administered systemically. Drugs delivered by the pulmonary route may or may not help treat systemic infection depending on the capacity to achieve adequate plasma concentration for therapy by this route of administration. In some instances, low or nondetectable plasma concentrations may be desirable in order to avoid systemic drug toxicities from prophylactic regimens or in patients with localized infections.

Lung delivery requires a particle size in the range of 1–5 μm (13,15). Therefore, mass distribution profiles of aerosols, which are used to derive mass median aerodynamic diameter (MMAD) and fine particle fraction (FPF), are of great interest to the investigators. The Product Quality Research Institute (PQRI) working group has expended significant effort in pursuing a profile comparator for aerosol particle size distribution (APSD) of pharmaceutical aerosols (16–18). The chi-square test has been proposed to serve as a statistical test for judging equivalence between reference and test profiles. However, this test was not recommended by the PQRI working group after intensive investigation (17). Principal component analysis (PCA) and orthogonal partial least square analysis (OPLS) have been employed frequently in the field of metabolomics mainly because of their ability to deal with very large data sets (19–21). However, their ability to compare APSD profiles has not been studied previously. PCA and OPLS may serve as powerful tools for individual judgment of equivalence of APSD profiles, while further effort is needed to study their potential as a general APSD profile comparator.

As part of the first phase of a clinical program to investigate aerosolized micafungin as a prophylactic agent for pulmonary infections, a comparison of different nebulizer systems is needed. The purpose of this study was to generate mass distribution profiles of aerosolized micafungin using two different nebulizers and to assess PCA and OPLS as methods for comparing the APSD profiles generated. Based on these data, three nebulizer candidates for clinical use will be evaluated in the future to determine the ideal device for micafungin delivery.

MATERIALS AND METHODS

Nebulizers and Drug

Acorn II (Marquest, Englewood, CO, USA) and LC Plus (Pari, Midlothian, VA, USA) nebulizers were driven by compressed air at a flow rate of 8 L/min. Micafungin (Astellas Pharma US, Deerfield, IL, USA), as supplied by the manufacturer, was diluted with 5 mL NaCl (0.9%) to a final concentration of approximately 10 mg/mL for nebulization. Drug analysis was performed by UV spectrophotometry at a maximum absorbance of 270 nm. Excipients present in micafungin include lactose and citric acid, which have no absorbance at this wavelength. A calibration curve was obtained with R^2=0.9995.

Particle Size Analysis

Mass median aerodynamic diameter (in micrometers) was calculated at two sampling periods using stage deposition data obtained from an Andersen cascade impactor (Ambient, 1ACFM nonviable, eight-stage sampler; Andersen, Smyrna, GA, USA) operated at a vacuum flow rate of 28.3 L/min. A short interval of sampling was used to avoid overloading the stages. Nebulizers were first operated for 2 min for Acorn II and 1 min for LC Plus and aerosol generated was directed to the cascade impactor. Then nebulization was stopped and nebulizers were stored at 4°C until the next operation. Both nebulizers were operated for another period of 4 min without directing the aerosol into the cascade impactor. After this, Acorn II and LC Plus nebulizers were operated again for another 2 and 1 min, respectively, with aerosols directed into a cascade impactor. All nebulization was carried out after the solution in the reservoir reached ambient temperature to eliminate temperature effect on mass distribution. FPF was calculated as the ratio of the mass deposited on stages below a certain cutoff size to the total amount of mass recovered. The geometric standard deviation (GSD) was calculated as the square root of the ratio of P_{84} (particle size under which 84% of the total mass was achieved in the cumulative mass distribution) to P_{16} (22).

Effect of Nebulization on the Concentration of Micafungin Sodium

At the beginning and after each 2-min nebulization interval, a 50 μL sample was withdrawn from the reservoir and stored in a 20-mL glass vial wrapped with aluminum foil. After collecting all samples, 14.95 mL of 20 mM KH_2PO_4 was added to each vial to bring the total volume up to 15 mL. The concentration of micafungin sodium was calculated from UV spectrophotometry data.

Statistical Analysis

Paired *t* test was used to compare MMAD from the two different sampling periods. OPLS was employed to compare percent mass distribution profiles within each nebulizer. PCA was employed to compare profiles between two nebulizers. Paired *t* test was carried out in Minitab 14.1 (Minitab). OPLS and PCA were carried out in SIMCA-P 11.5 (Umetrics). Data were pretreated with mean-centering and unit-variance scaling before executing OPLS and PCA.

RESULTS

Mass Distribution from Two Nebulizers

The percent mass APSD profiles of micafungin sodium are shown in Fig. 1. Very little drug was deposited in the throat and inlet during sampling. Most deposition occurred on stage 5 (1.1 μm) and stage 4 (2.1 μm) for both Acorn II and LC Plus nebulizers. A significant difference between APSD profiles generated from the two nebulizers was observed visually. Compared with the profile from the Acorn II nebulizer, there was significantly greater mass deposited on stage 2 (4.7 μm) and stage 3 (3.3 μm) with the LC Plus nebulizer. Profile change from the first sampling period to the second was also observed.

Effect of Nebulizers and Nebulization Time on Aerosol Property

Mass median aerodynamic diameter, GSD, and FPF were all calculated and are tabulated in Table I. The mass

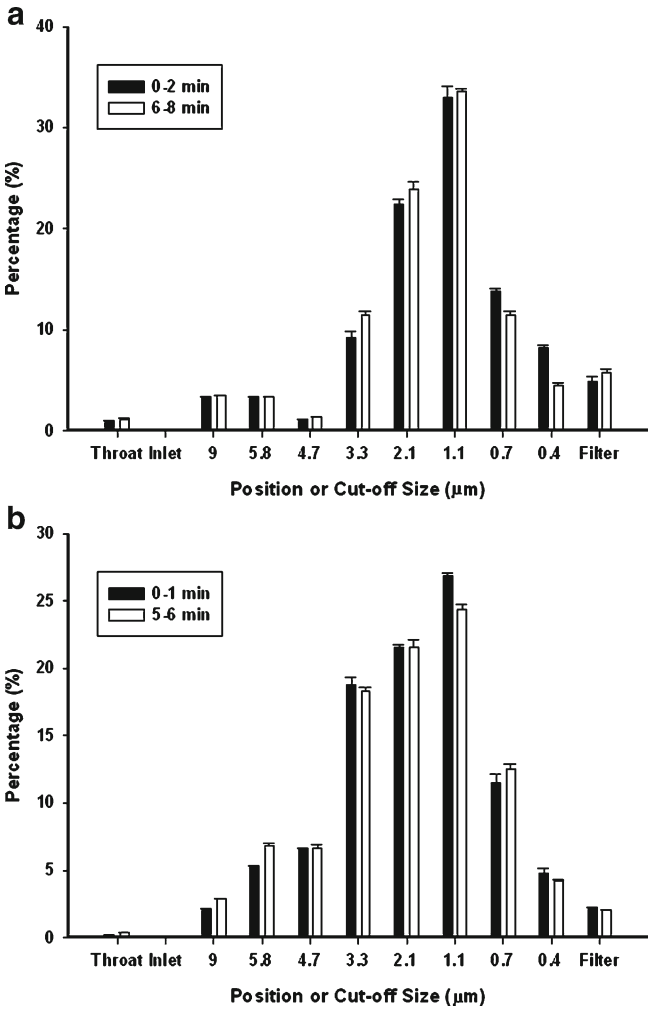

Fig. 1. Percent mass APSD of micafungin sodium measured by Andersen cascade impactor. **a** Micafungin was delivered *via* Acorn II nebulizer and measured at 0–2 and 6–8 min, respectively. **b** Micafungin was delivered *via* LC Plus nebulizer and measured at 0–1 and 5–6 min, respectively. Each bar represents the mean±SEM (n=3)

deposited in the cascade impactor follows log-normal distribution and GSD was thus calculated. The MMAD derived from Acorn II was smaller than that derived from LC Plus. The MMAD from the first nebulization was smaller than that from the second nebulization for both Acorn II and LC Plus nebulizers. This observation is statistically different by paired t test (Acorn II, p=0.015; LC Plus, p=0.011). Paired t test was justified for this comparison since the experiment was

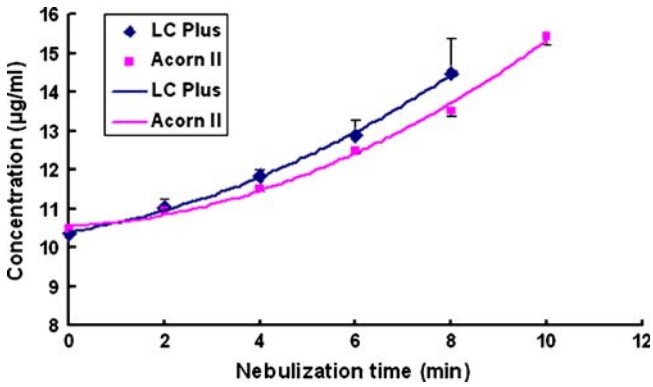

Fig. 2. Effects of nebulization time on the concentration of micafungin sodium. The concentration of micafungin sodium in the reservoir was measured by UV spectrophotometry at different time points after nebulization. Each data point represents the mean±SEM (n=3). A quadratic function was employed to fit in data points

designed in such a way that both the first and second nebulizations were carried out from one parent solution and the same nebulizer. $FPF_{3.3}$ (%<3.3 μm) for the Acorn II is significantly higher than that for the LC Plus. However, this difference was not observed for $FPF_{5.8}$ (%<5.8 μm).

Effect of Nebulization Time on Drug Concentration

The concentration of micafungin sodium increased continuously with duration of nebulization for both nebulizers (Fig. 2). The concentration in the LC Plus increased more rapidly than that of the Acorn II nebulizer. A quadratic function was employed to fit data points with R^2>0.99 for both regression analyses. Compared with the initial concentration, the final concentration of micafungin sodium increased by 48% with the Acorn II nebulizer and by 40% with the LC Plus nebulizer. From the first to the second sampling period, the concentration of micafungin sodium increased by 21% with the Acorn II nebulizer compared with 20% with the LC Plus nebulizer.

Multivariate Data Analysis for Profile Comparison

OPLS was used for profile comparison within each nebulizer. The goodness of OPLS model fit (R^2) is 0.889 for Acorn II nebulizer and 0.921 for LC Plus nebulizer. The predictive power of OPLS model (Q^2) is 0.807 for Acorn II and 0.808 for LC Plus nebulizer. Each data point in the scores plot (Fig. 3) represents a percent mass distribution profile obtained from a single experiment. As shown, mass profiles of

Table I. Effect of nebulizers and nebulization time on particle size and distribution (MMAD, μm, and GSD) and fine particle fraction (FPF% <3.3 μm, FPF%<5.8 μm)

	Acorn II (0–2 min)	Acorn II (6–8 min)	LC Plus (0–1 min)	LC Plus (5–6 min)
MMAD (μm)	1.67±0.05	1.77±0.04	2.09±0.01	2.20±0.01
GSD	2.28±0.01	2.27±0.03	2.13±0.02	2.18±0.01
FPF (%<3.3 μm)	82.3±2.1	79.1±1.1	67.0±0.7	64.8±0.8
FPF (%<5.8 μm)	92.5±0.8	91.9±0.3	92.3±0.1	91.1±1.3

n=3, mean±SEM
MMAD mass median aerodynamic diameter, *GSD* geometric standard deviation, *FPF* fine particle fraction

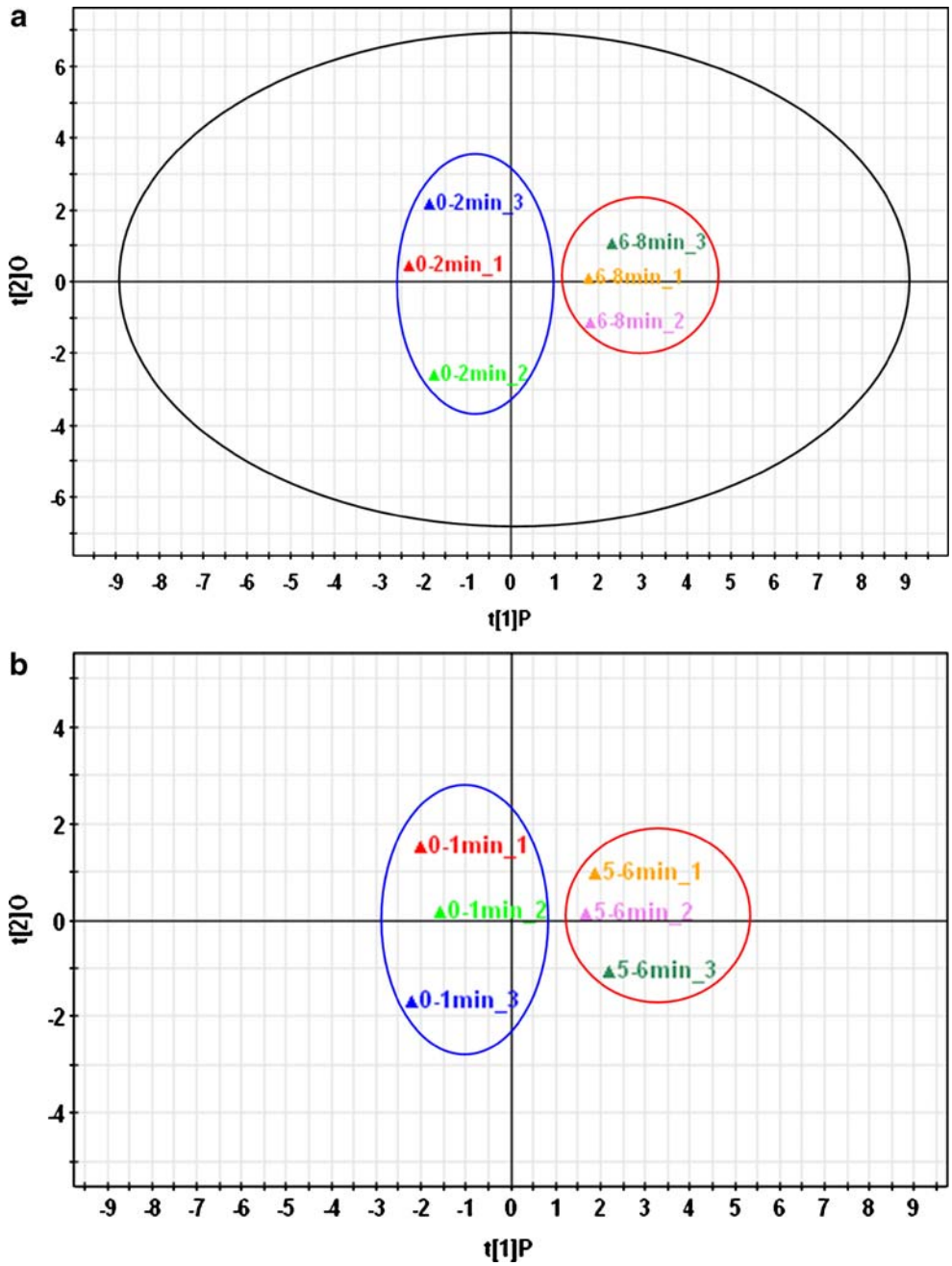

Fig. 3. Scores plot from OPLS analysis of percent mass APSD profiles obtained from two nebulization periods. **a** Micafungin was nebulized by Acorn II nebulizer. **b** Micafungin was nebulized by LC Plus nebulizer. The *blue ellipse* includes three data points from the first nebulization period and the *red ellipse* includes three data points from the second nebulization period

the first nebulization period were easily discriminated from that of the second period as marked by the different colored ellipses.

The loadings plot (Fig. 4) suggested variables which were responsible for the patterns seen in the scores plot. As shown in Fig. 4a, deposition on stages 7 and 6 (0.4 and 0.7 μm, respectively) and stages 2, 3, 0, and 4 (4.7, 3.3, 9.0, and 2.1 μm, respectively) account for the separation of two sets of profiles (first *vs.* second nebulization) for the Acorn II nebulizer. Deposition on stage 5 (1.1 μm), filter, and stage 1, 0 (5.8 and 9.0 μm) account for the separation of two sets of profiles (first *vs.* second nebulization) for the LC Plus nebulizer.

The relative importance and confidence interval for each variable that contributed to the profile separation is plotted in Fig. 5. Variables with the largest absolute mean value and the smallest confidence intervals are most important, accounting for profile separation. A conclusion regarding which variables were responsible for the patterns seen in the scores plot could also be reached from the contribution plot.

We further studied the APSD profiles by pooling all of the profiles from Acorn II and LC Plus nebulizers. PCA was used to find pattern in these profiles (Fig. 6). Without *a priori* knowledge of the identity of each profile, PCA clearly

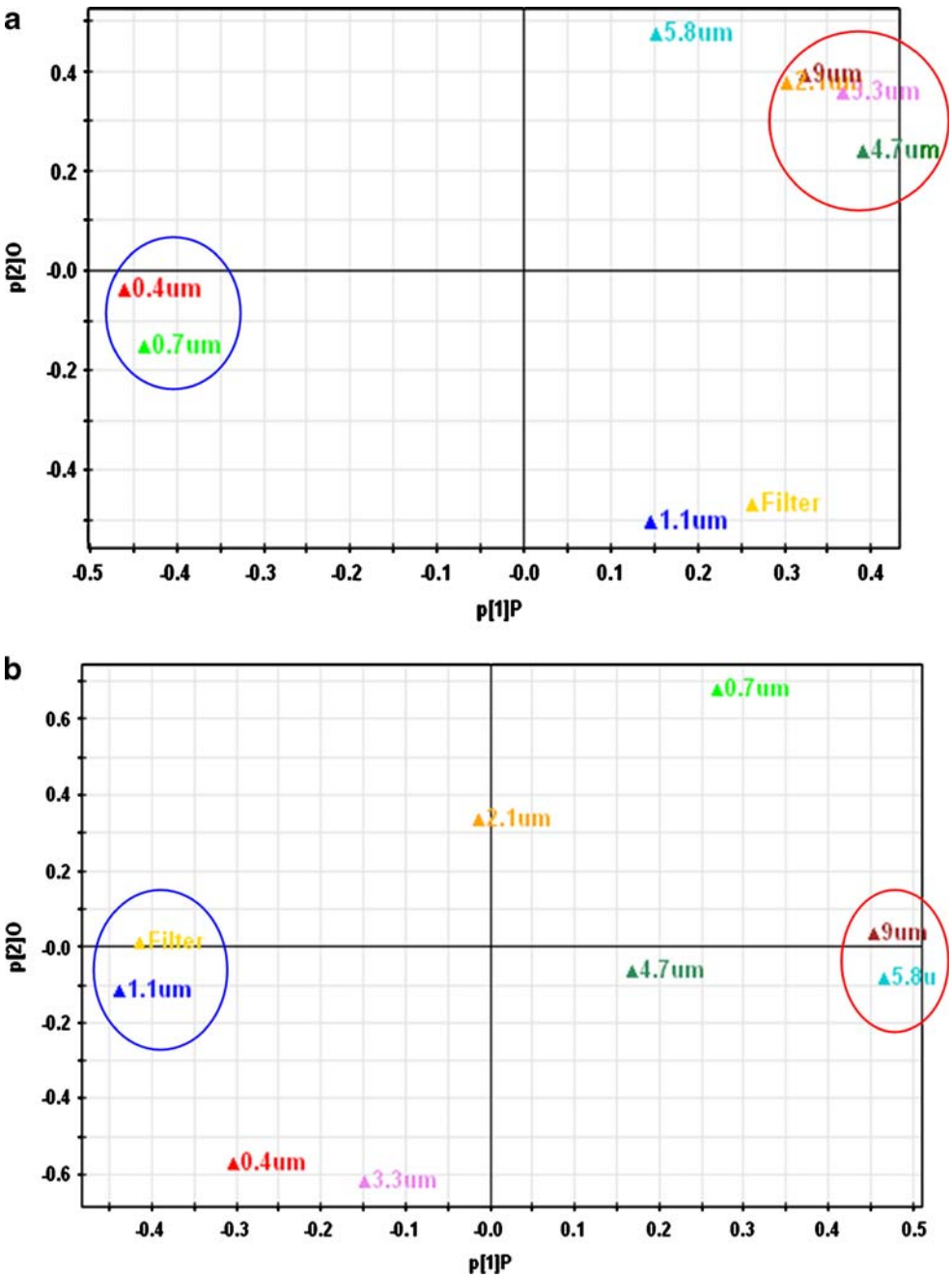

Fig. 4. Loadings plot from OPLS analysis of percent mass APSD profiles obtained from two nebulization periods. **a** Micafungin was nebulized by Acorn II nebulizer. **b** Micafungin was nebulized by LC Plus nebulizer. *Blue* and *red ellipses* mark the cascade impactor positions where mass deposition of micafungin sodium changed significantly from the first nebulization period to the second nebulization period

discriminated between them. A pattern could be seen with profiles from LC Plus nebulizers clustered to the left side of the scores plot (Fig. 6a) and profiles from Acorn II nebulizers located to the right side. Furthermore, a separation could be seen for profiles from Acorn II nebulizer of different nebulization periods. No such separation could be seen for LC Plus nebulizer. The loadings plot (Fig. 6b) showed the influential variables that contributed to the pattern seen in the scores plot (Fig. 6a).

DISCUSSION

Intravenous infusion is the only route that has been approved for the administration of micafungin to patients. To our knowledge, there is no published data on the use of aerosolized micafungin for the prevention or treatment of invasive fungal infection. This is the first description of the characteristics of a micafungin aerosol. Aerosolized micafungin may be particularly attractive for several reasons: (1)

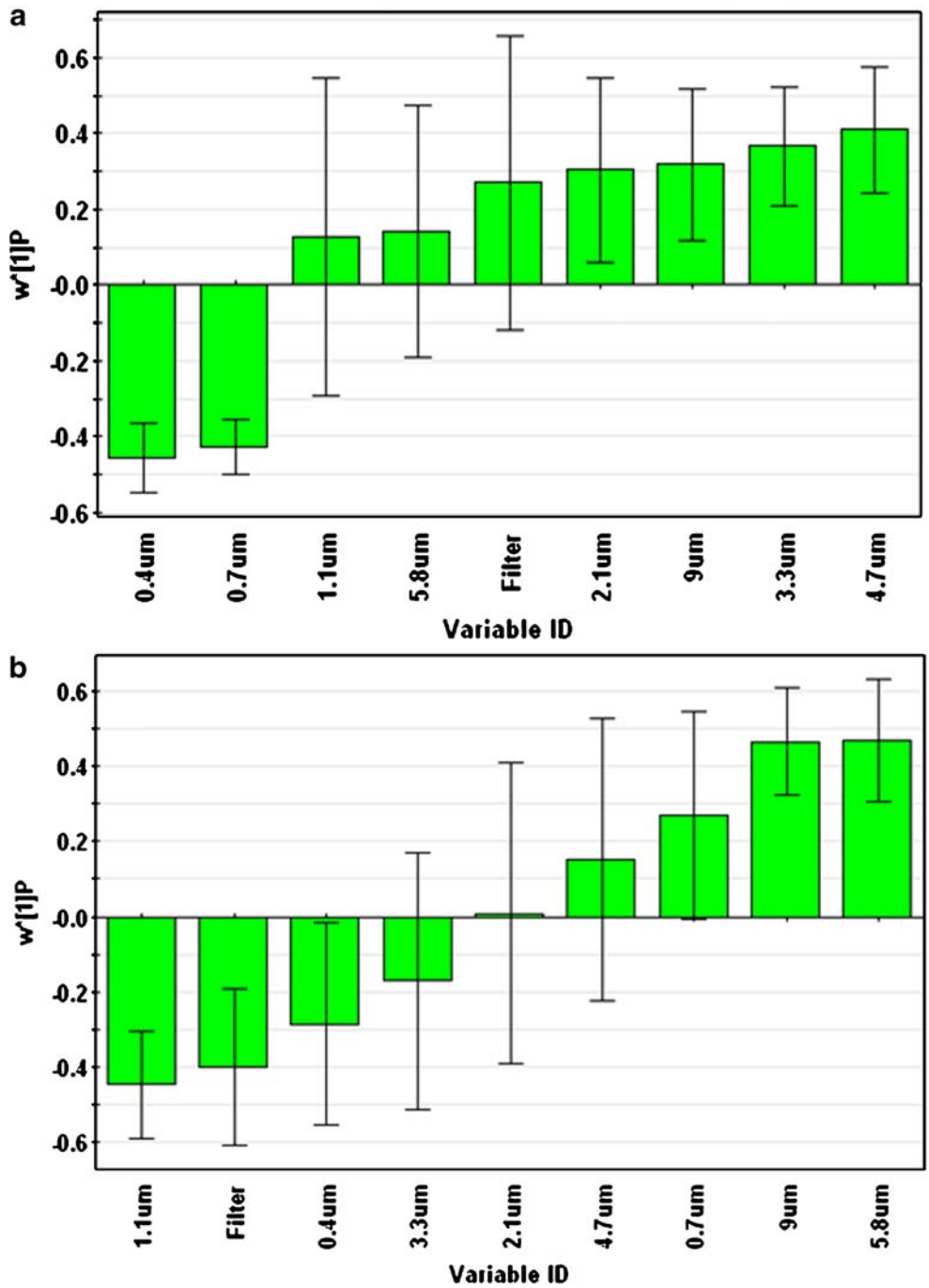

Fig. 5. Contribution plot from OPLS analysis of percent mass APSD profiles obtained from two nebulization periods. Error bar represents the confidence interval

targeting micafungin to the lung may result in improved efficacy and reduced toxicity; (2) aerosolized micafungin may potentially attain higher concentrations in the epithelial lining fluid and upper airways than can be achieved by intravenous infusion; (3) targeted pulmonary delivery *via* nebulization may alleviate the need for intravenous infusion; and (4) the duration of administration may be significantly shortened given the minimum 1 h intravenous infusion time for this agent.

We noticed that there was a small increase in the MMAD from the first nebulization period to the second for both nebulizers. This modest increase in the MMAD may be explained by the increased concentration of micafungin, which results from evaporation during nebulization. Large surface areas were formed by nebulization, which greatly accelerates evaporation by mass transfer from aqueous phase to gas phase (23,24). The concentration change of micafungin between the two nebulization periods corresponds relatively well to the change in MMAD based on simple calculations. Although this small increase in MMAD was statistically significant, it may not be clinically important since both MMADs fall within the desired range. $FPF_{5.8}$ for both nebulizers exceeded 90%, which indicates that a significant

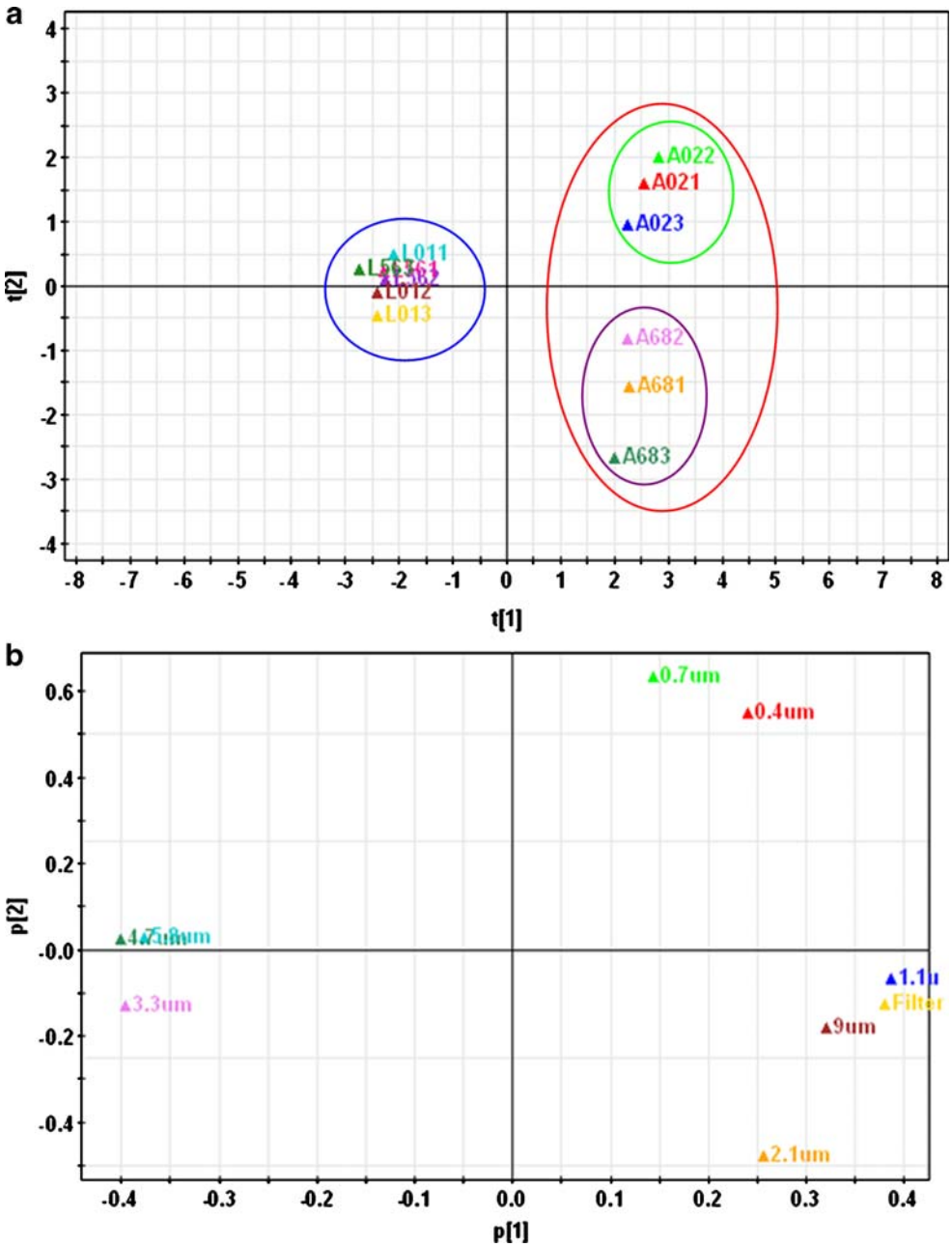

Fig. 6. PCA of percent mass APSD profiles from Acorn II and LC Plus nebulizers. **a** Scores plot with the first two components (*A* indicates Acorn II nebulizer; *L* LC Plus; *first two digits* nebulization period; *last digit* experiment number). **b** Loadings plot with the first two components. Variables that contribute to the separation in the scores plot could be found correspondingly in the loadings plot

proportion of nebulized droplets can reach peripheral lungs. The flow rate used in these studies was in the recommended ranges for each nebulizer; no efforts were made to optimize delivery conditions for the nebulizers.

Although the paired *t* test could be used to detect the differences in MMAD, this parameter is a derivative from mass distribution profiles. Important information could be lost if MMAD is the only parameter assessed. A profile comparison method should be used to consider APSDs as a whole rather than focusing on MMAD. Candidate methods include *t* test, chi-square test (16), and PCA. Multiple *t* tests with Bonferroni correction may be considered for profile comparison. However, multiple comparisons will result in inflation of type I error, the error of rejecting a null hypothesis when it is actually true. Bonferroni correction may control the overall error rate; however, it was considered too stringent to reject the null hypothesis.

Other correction methods may also be employed, although there is currently no consensus about which correction method is the best. Chi-square test has been intensively investigated as a tool for comparison of APSD of aerosolized drug formulations. However, it was not recommended for APSD profile comparison for several reasons. The main concern for the chi-square test is that it

fails to detect the difference among profiles when a difference does exist (16–18). In this study, we propose another method for APSD profile comparison for the following reasons: (1) APSD profile is multidimensional, having approximately ten predictive variables; (2) the masses deposited on each stage are not totally independent (16,17); and (3) the tool should not only be able to detect the difference, but also pinpoint the origin of the difference.

OPLS and PCA can reduce multiple dimensions into one or two major components. The issue of covariance among predictive variables could also be solved by these two methods since new components are all orthogonal. Moreover, the scores plot of PCA and OPLS could provide an overall pattern of APSD profiles studied. If a particular pattern was observed, the influential variables that contribute to the pattern could be found in the corresponding loadings plot.

In the present study, we performed OPLS for APSD profile comparison within each nebulizer. A pattern (the separation of profiles from two nebulization periods) was observed in the scores plot. Furthermore, this separation can be explained by the loading and contribution plots, indicating that greater drug was deposited on plates with larger cutoff sizes and reduced drug deposition on plates with smaller cutoff sizes from the first nebulization period to the second one. Although several stages contributed to the separation, we should first focus on the most influential stages where confidence is highest.

PCA was performed for APSD profiles comparison between two nebulizers. PCA is different from OPLS in that *a priori* knowledge of the identity of the profiles is not employed while patterns are assessed. It is usually a good practice to perform PCA before proceeding to OPLS. From Fig. 6, a clear pattern could be seen. Profiles from the Acorn II nebulizer were completely separated from the profiles of the LC Plus nebulizer. And the separation among profiles from the two nebulization periods of Acorn II nebulizer could also be seen. In contrast, no such separation was observed for LC Plus nebulizer. A possible explanation is that PCA places the first component onto between-nebulizer difference (largest difference) and the second component onto within-nebulizer difference of Acorn II nebulizer (second largest difference). However, the within-nebulizer difference of LC Plus nebulizer is relatively small compared to the above differences and may be represented by a successive component, which was not shown in the figure.

One potential application for PCA and OPLS is to evaluate cascade impactor profiles in general for pharmaceutical aerosols. However, one concern regarding PCA and OPLS is that they are not discriminating statistical tests but analytical tools. They will not provide a "yes" or "no" answer to questions of comparability or equivalence of profiles. However, certain parameters derived from these analyses may serve as criteria for comparability or equivalence evaluation.

CONCLUSION

Aerosol delivery of drug provides a unique opportunity in the prevention and/or treatment of invasive fungal infections. Pulmonary delivery of antifungal agents may increase local concentrations of the drug at the site where many fungal pathogens gain entry into the body and establish invasive infection. In the present study, both Acorn II and LC Plus are suitable nebulizers for pulmonary delivery of micafungin in terms of FPF and MMAD. However, a much greater understanding of nebulizers, efficiency of delivery, and local drug deposition is required for this approach to gain general acceptance. Although their potential use as an APSD profile comparator requires much greater study before their application can be generally recommended, multivariate data analysis such as PCA and OPLS are good pattern recognition tools and offer scientists a useful mechanism by which to evaluate APSD profiles generated with different nebulizers.

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REFERENCES

1. J. J. Vehreschild, and O.A. Cornely. Micafungin sodium, the second of the echinocandin class of antifungals: theory and practice. *Future Microbiol.* **1**:161–170 (2006).
2. Astellas Pharma US. Mycamine (micafungin sodium) package insert. Deerfield, IL (2005).
3. H. Mikamo, Y. Sato, and T. Tamaya. *In vitro* antifungal activity of FK463, a new water-soluble echinocandin-like lipopeptide. *J. Antimicrob. Chemother.* **46**:485–487 (2000).
4. P. H. Chandrasekar, and J. D. Sobel. Micafungin: a new echinocandin. *Clin. Infect. Dis.* **42**:1171–1178 (2006).
5. J. M. Joseph, R. Jain, and L. H. Danziger. Micafungin: a new echinocandin antifungal. *Pharmacotherapy.* **27**:53–67 (2007).
6. T. Nakai, J. Uno, K. Otomo, F. Ikeda, S. Tawara, T. Goto, K. Nishimura, and M. Miyaji. *In vitro* activity of FK463, a novel lipopeptide antifungal agent, against a variety of clinically important molds. *Chemotherapy.* **48**:78–81 (2002).
7. V. Petraitis, R. Petraitiene, A. H. Groll, K. Roussillon, M. Hemmings, C. A. Lyman, T. Sein, J. Bacher, I. Bekersky, and T. J. Walsh. Comparative antifungal activities and plasma pharmacokinetics of micafungin (FK463) against disseminated candidiasis and invasive pulmonary aspergillosis in persistently neutropenic rabbits. *Antimicrob. Agents Chemother.* **46**:1857–1869 (2002).
8. T. Nakai, J. Uno, F. Ikeda, S. Tawara, K. Nishimura, and M. Miyaji. *In vitro* antifungal activity of Micafungin (FK463) against dimorphic fungi: comparison of yeast-like and mycelial forms. *Antimicrob. Agents Chemother.* **47**:1376–1281 (2003).
9. K. Tabata, M. Katashima, A. Kawamura, Y. Tanigawara, and K. Sunagawa. Linear pharmacokinetics of micafungin and its active metabolites in Japanese pediatric patients with fungal infections. *Biol. Pharm. Bull.* **29**:1706–1711 (2006).
10. M. F. Hebert, H. E. Smith, T. C. Marbury, S. K. Swan, W. B. Smith, R. W. Townsend, D. Buell, J. Keirns, and I. Bekersky. Pharmacokinetics of micafungin in healthy volunteers, volunteers with moderate liver disease, and volunteers with renal dysfunction. *J. Clin. Pharmacol.* **45**:1145–1152 (2005).
11. T. Niwa, Y. Yokota, A. Tokunaga, Y. Yamato, A. Kagayama, T. Fujiwara, J. Hatakeyama, M. Anezaki, Y. Ohtsuka, and A. Takagi. Tissue distribution after intravenous dosing of micafungin, an antifungal drug, to rats. *Biol. Pharm. Bull.* **27**:1154–1156 (2004).
12. A. H. Groll, D. Mickiene, V. Petraitis, R. Petraitiene, K. H. Ibrahim, S. C. Piscitelli, I. Bekersky, and T. J. Walsh. Compartmental pharmacokinetics and tissue distribution of the antifungal echinocandin lipopeptide micafungin (FK463) in rabbits. *Antimicrob. Agents Chemother.* **45**:3322–3327 (2001).

13. A. J. Hickey. Pulmonary drug delivery—pharmaceutical chemistry and aerosol technology. In B. Wang, T. Siahaan, and R. Soltero (eds.), *Drug Delivery: Principles and Applications*, Wiley, New York, 2005, pp. 341–361.
14. A. J. Hickey, D. Lu, E. D. Ashley, and J. Stout. Inhaled azithromycin therapy. *J. Aerosol. Med.* **19**:54–60 (2006).
15. A. J. Hickey. Summary of common approaches to pharmaceutical aerosol administration. In A. J. Hickey (ed.), *Pharmaceutical Inhalation Aerosol Technology*, 2nd ed., Marcel Dekker, New York, 2004, pp. 385–421.
16. D. Christopher, W. P. Adams, D. S. Lee, B. Morgan, Z. Pan, G. J. Singh, Y. Tsong, and S. Lyapustina. Product Quality Research Institute evaluation of cascade impactor profiles of pharmaceutical aerosols, part 2: evaluation of a method for determining equivalence. *AAPS PharmSciTech.* **8**:5 (2007).
17. D. Christopher, W. Adams, A. Amann, C. Bertha, P. R. Byron, W. Doub, C. Dunbar, W. Hauck, S. Lyapustina, J. Mitchell, B. Morgan, S. Nichols, Z. Pan, G. J. Singh, T. Tougas, Y. Tsong, R. Wolff, and B. Wyka. Product Quality Research Institute evaluation of cascade impactor profiles of pharmaceutical aerosols, part 3: final report on a statistical procedure for determining equivalence. *AAPS PharmSciTech.* **8**:E90 (2007).
18. W. P. Adams, D. Christopher, D. S. Lee, B. Morgan, Z. Pan, G. J. Singh, Y. Tsong, and S. Lyapustina. Product Quality Research Institute evaluation of cascade impactor profiles of pharmaceutical aerosols, part 1: background for a statistical method. *AAPS PharmSciTech.* **8**:4 (2007).
19. R. S. Plumb, M. D. Jones, P. D. Rainville, and J. K. Nicholson. A rapid simple approach to screening pharmaceutical products using ultra-performance LC coupled to time-of-flight mass spectrometry and pattern recognition. *J. Chromatogr. Sci.* **46**:193–198 (2008).
20. J. C. Lindon, E. Holmes, and J. K. Nicholson. Metabonomics in pharmaceutical R&D. *FEBS J.* **274**:1140–1151 (2007).
21. S. Rochfort. Metabolomics reviewed: a new "omics" platform technology for systems biology and implications for natural products research. *J. Nat. Prod.* **68**:1813–1820 (2005).
22. The United States Pharmacopeial, General Chapter <601> Aerosols, Nasal Sprays, Metered-Dose Inhalers, and Dry Powder Inhalers. (2008).
23. R. W. Niven, M. Speer, and H. Schreier. Nebulization of liposomes. II. The effects of size and modeling of solute release profiles. *Pharm. Res.* **8**:217–221 (1991).
24. R. W. Niven, T. M. Carvajal, and H. Schreier. Nebulization of liposomes. III. The effects of operating conditions and local environment. *Pharm. Res.* **9**:515–520 (1991).