Bulk Composition Analysis of Microparticle-based Pharmaceutical Dosage Forms by Macro-Raman Spectroscopy

Hui Wang[1], Lisa Williams[2], Susan Hoe[2], David Lechuga Ballesteros[2], and Reinhard Vehring[1, 2]

[1]University of Alberta, Edmonton, AB, Canada

[2]Pearl Therapeutics, Inc., Redwood City, CA, USA

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INTRODUCTION

Bulk composition analysis of pharmaceutical dosage forms is important for quality control during the shelf life of the product, mainly because of the occurrence of sub-standard drugs [1], and most importantly, for monitoring potential transformations of actives and excipients into other forms or states [2]. However, quantification errors caused by non-representative sampling have been reported as an important error source, especially for sensitive techniques analyzing very small samples, e.g., micro-Raman systems with spatial resolution in the micrometer scale [3]. For Raman spectroscopy, larger scattering volumes have been intentionally pursued using various methods [4, 5]. A quantitative description of the minimum sample volume required for representative sampling of microparticle based powder samples is presented here.

MATERIALS AND METHODS

Commercial respirable dosage forms Seretide® 50 Evohaler® and Seretide® Accuhaler® (GSK) were used in this study. Pressurized metered dose inhaler (pMDI) product Seretide® 50 Evohaler® contains 50 µg fluticasone propionate (FP) and 25 µg salmeterol xinafoate (SX) per dose. Seretide® 100, 250, and 500 Accuhaler® are lactose

carrier-based dry powder inhaler (DPI) products containing different strengths of salmeterol.

Particle size distributions of non-respirable lactose carrier particles in the tested dry powder inhaler formulations were measured using a laser diffraction system (HELOS BF, Sympatec GmbH, Clausthal-Zellerfeld, Germany) with an attached powder disperser (OASIS/M, Sympatec GmbH, Clausthal-Zellerfeld, Germany). The measured lactose particle sizes are used as input in the following simulation. Particle sizes of active FP and SX have been well characterized in literature and were also used in the following model [6].

A stochastic model was developed to simulate the random sampling process of multi-component micro-particle based powder samples, represented by the commercial pMDI and DPI powder samples in this study. Briefly, the simulation began with a very large sample volume, which was filled with randomly-generated virtual component particles according to input component characteristics. When the volume was full, the relative error of each component was calculated and compared with a specified error tolerance. If the error was within the tolerance, a smaller sample volume was then simulated similarly and this process was repeated iteratively until the error exceeded the specified error tolerance. Each simulation returned one minimum sample volume. Simulations were repeated 100 times and the cumulative distribution of the simulated minimum sample volumes for each sample were then plotted and fitted by a lognormal distribution using the least square method. The sampling error of a specified sample volume can also be estimated using this model.

A custom designed dispersive macro-Raman system [7], using a 0.16 µL conical cavity as a sample holder, was used to quantitatively evaluate the compositions of respirable powder samples extracted from the three DPI devices. However, the

detected sample volume was restricted by the optical magnification of the setup, spectrograph slit width, and recorded sensor area, which gave a lower limit of scattering volume of 0.004 µL. The effective sample volume is between 0.004 µL and 0.16 µL due to multiple reflections and homogenizations of both excitation light and Raman signals in the cavity. Each sample was independently measured five times. The spectral contributions of each component in the composite sample spectra were separated by deconvolution. The deconvoluted spectral intensities were then converted into composition information according to a derived calibration curve [8]. Errors of quantitative measurements were compared with errors predicted by the simulation model.

RESULTS AND DISCUSSION

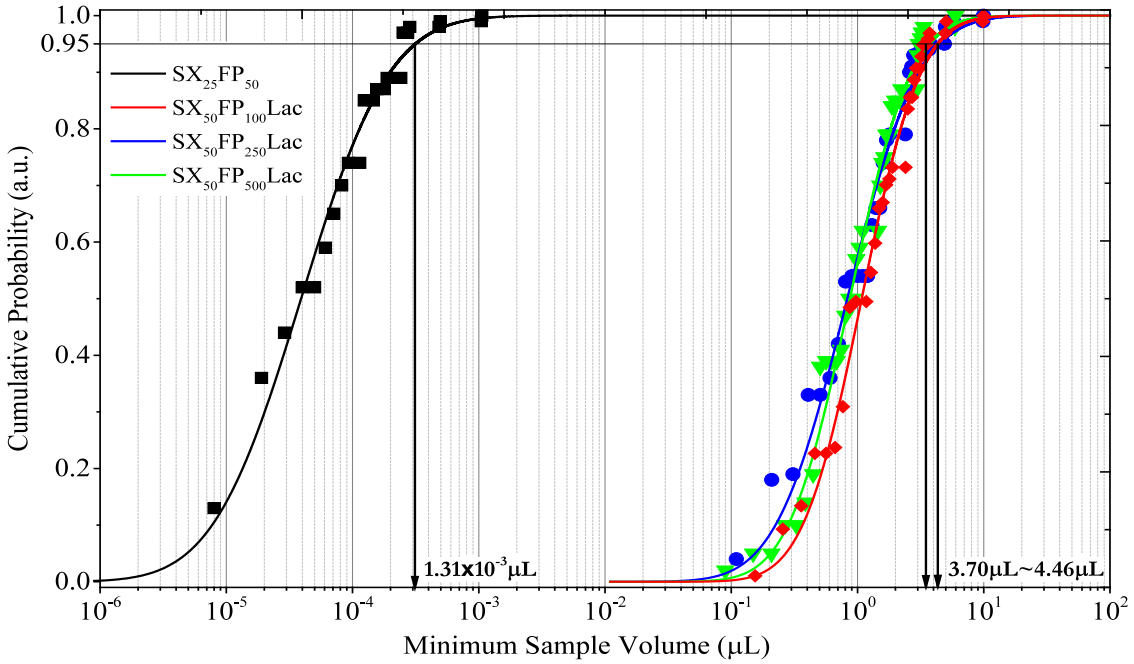

Figure 1. Predicted minimum sample volume distributions of commercial pMDI and DPI

Cumulative distributions of predicted minimum sample volumes for both the carrier-free pMDI product and the lactose-based DPI products are presented in Figure 1. For

carrier-free Seretide® 50 Evohaler® consisting of respirable sized particles only, the minimum sample volume required, for less than 3% relative error with a 95% confidence after 5 independent sampling events, is on the order of 10^{-3} µL. However, the minimum sample volume for the carrier-drug Seretide® Accuhaler® products is more than three orders of magnitude greater, in the microliter range. This is mainly due to the dominant presence of large non-respirable lactose particles and resultant low-concentration of active fines, which reduces the number of particles that can be sampled per unit volume, and thus requires a larger volume to achieve representative sampling. In both cases, the minimum sample volumes correspond to millions of particles, which would be very difficult to detect simultaneously or consecutively with micro-Raman systems, because typically their scattering volumes are less than 10^{-6} µL. Another limitation of micro-Raman spectroscopy for bulk sample analysis is the slow rate of data acquisition due to the low tolerable laser power and small number of analyzed Raman scatterers.

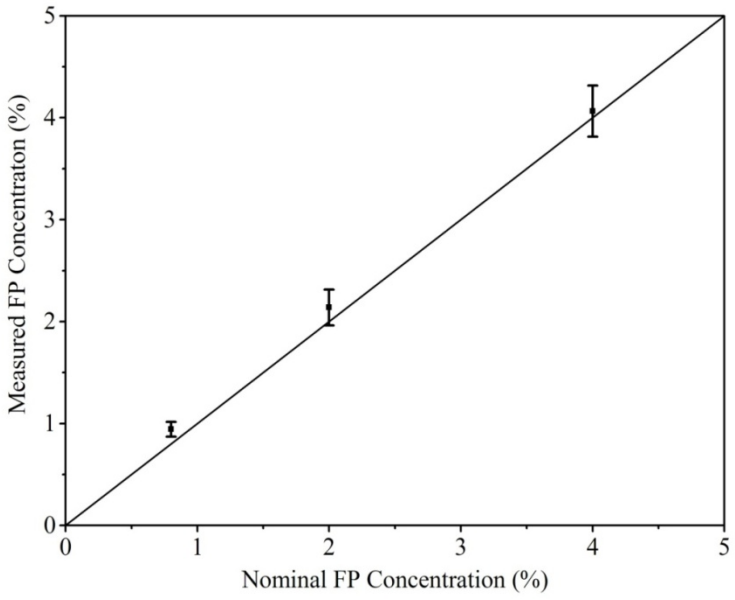

Figure 2. Measured FP mass fractions in comparison with nominal compositions

Quantitative analysis results of the three DPI samples are presented in Figure 2 with measured FP compositions plotted against their nominal values. Error source

distributions of the measurements including instrument variations, quantification methodology error, and sampling error are analyzed and listed in Table 1. Sampling errors based on a sample volume of 0.16 µL were estimated using the same simulation model. It is apparent that the first sample with the lowest strength of FP has a mean relative error of 17.5% based on the five independent measurements and it is larger than the estimated error. This is because the effective Raman scattering volume of the instrument is smaller than 0.16 µL, as described above, which may give rise to larger sampling errors for this particular powder sample.

Table 1. Error distributions of macro-Raman quantitative analysis

Formulation	Nominal FP mass fraction %	Measured FP mass fraction (±*S.D.*) %	Relative Error %	Relative Error % (±)		
				Spectral noise and imperfect reference	Predicted Sampling error for 0.16µL (±*S.D.*)	Quantification method
50µg SX + 100µg FP + Lactose	0.8	0.94(±0.07)	17.5±8.8	2.4	4.7(±4.6)	7.4
50µg SX + 250µg FP + Lactose	2.0	2.14(±0.18)	7.0±9.0	2.0	4.3(±3.8)	8.4
50µg SX + 500µg FP + Lactose	4.0	4.07(±0.25)	1.8±6.3	1.8	6.0(±5.6)	6.1

CONCLUSIONS

Macro-Raman spectroscopy with a large sample volume is suitable for representative composition analysis of bulk powder samples. To achieve less than 3% relative error tolerance with high confidence, the minimum sample volume predicted by the stochastic model for carrier-free metered dose inhaler or dry powder inhaler products is on the order of 10^{-3} µL, containing millions of particles. However, for dosage forms containing non-respirable large carrier particles and fine components in extremely low concentrations, the required sample volume increases to several microliters. The

results are not only instructive for macro-Raman sample preparation of respirable dosage forms but also applicable to any other technique measuring bulk compositions of micro-particle based powder samples. Because the model assumes a perfectly mixed sample, inhomogeneous samples require even larger sample volumes.

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