Resolving a few misconceptions when analyzing data from inhaler particle size distribution measurements

Commonly encountered errors to avoid

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Introduction

Particle/droplet size distributions from orally inhaled and nasal drug products (OINDPs) are critical quality attributes and part of the product quality target performance profile (QTPP) in the quality control environment [1-3]. Such measurements also support clinical programs during product development [4]. It is necessary to know the particle size distribution scaled in terms of aerodynamic diameter (a.k.a. aerodynamic particle size distribution or APSD) for orally inhaled products (OIPs), because the deposition profile of the inhaled airborne particles in the lungs is critically dependent upon aerodynamic diameter rather than physical (geometric) diameter [5]. However, for nasal-inhaled products, comprising nasal pressurized metered dose inhalers (npMDIs) and aqueous nasal sprays (NSs), the size distribution can be based on physical (geometric) diameter because these particles are not subjected to significant aerodynamic size fractionation before entering the nasal cavity. Volume median diameters are typically found to be in the range of 30 to 120 µm [6]. The concept of aerodynamic particle size with these products is only important when it is necessary to ascertain the typically small mass fraction contained in droplets finer than about 15 µm aerodynamic diameter that may penetrate beyond the nasopharynx and deposit in the airways of the lungs [7].

The purpose of this article is to identify and resolve a few important misconceptions that from-time-to-time have appeared concerning the analysis of particle size data. A key objective is to guide the newcomer to the field, as well as those with familiarity of the topic. The focus is primarily on multi-stage cascade impaction, which is the primary technique for OIP APSD measurement [1, 2]. However, attention is also paid to the analysis of size distribution data from time-of-flight (TOF) analyzers and laser diffractometer (LD) instruments that are also encountered in the evaluation of inhaler performance, particularly the latter for the assessment of nasal products (NPs). Recently, the Joint Statistics and Aerosols sub-committees of the United States Pharmacopeial Convention (USP) published a Stimulus to Revision article in which, for the first time, clear guidance has been provided in the analysis of such APSD data [8]. The approach taken can be adapted to the analysis of size distribution data from other techniques, in particular laser diffractometry (LD).

Misconception awareness in particle sizing data

Misconception 1: Impactor-sized mass (ISM) always includes data from all impactor stages.

ISM is defined as the total mass of active pharmaceutical ingredient (API) contained in the *sized* portion of the sampled OIP aerosol, and therefore encompasses the entire measurable APSD from the inhaler. It is desirable to reduce the large amount of information available from the APSD. This process often involves reporting the maximum and minimum size limits together with ISM and a measure of central tendency, typically the mass median aerodynamic diameter (MMAD). Further, it is useful to know a measure of APSD spread, and this parameter is often reported as the geometric standard deviation (GSD), or in more general terms, the span calculated as the difference in sizes between the 10th and 90th percen-

tiles of the ISM, normalized by dividing this difference by the size corresponding to the MMAD. ISM does not account for the non-sized mass of aerosol emitted from the inhaler (Figure 1), that may comprise a significant mass of API that is retained by the inhaler mouthpiece/facemask, recovered from the inlet (usually the USP/PhEur design in product quality control testing) and from the interior of the pre-separator (PS), if used. It should be noted that ISM includes the mass of API recovered on a final filter if present in the cascade impactor (standard equipment for the Andersen Cascade Impactor (ACI) and an option for the Next Generation Impactor (NGI)).

A misconception can arise when ISM is estimated as the sum of API mass on *all* impactor stages. This estimate is only accurate when the PS is present and the cut-point size, defined as the aerodynamic diameter at which this component captures incoming particles with 50% efficiency, has been established by formal calibration with particles of known aerodynamic size (see the upper configuration in Figure 1A). Such a procedure was undertaken with monodisperse particles for the PS of the NGI at 30, 60 and 100 L/min, as part of its archival calibration [9]. In contrast, several calibrations of the Andersen 8-stage impactor have been published from-time-to-time [10-13]. However, where a PS was evaluated, these calibrations were not archival in nature, in that, in the case of the NGI, the nozzle dimensions of this component, as well as the size-fractionating stages, were manufactured to be as close as possible to nominal sizes. It follows that, given the lack of certainty as to the cut-point sizes of the three PS options currently available for use with the ACI at 28.3, 60 and 90 L/min, estimations of ISM should exclude the first sizing stage if calculated from measurements made with either this impactor with or without its PS or the NGI without PS (Figure 1B). In contrast, ISM derived from NGI measurements with its PS, as is the norm for most dry powder inhalers (DPIs), does include the mass recovered from stage 1 (Figure 1A). It is self-evident that, though not normally done, the option exists to test pressurized metered dose inhalers (pMDIs) and soft mist inhalers by NGI with its PS, whereby an additional data point can be gained as the result of having the upper size limit for stage 1 defined.

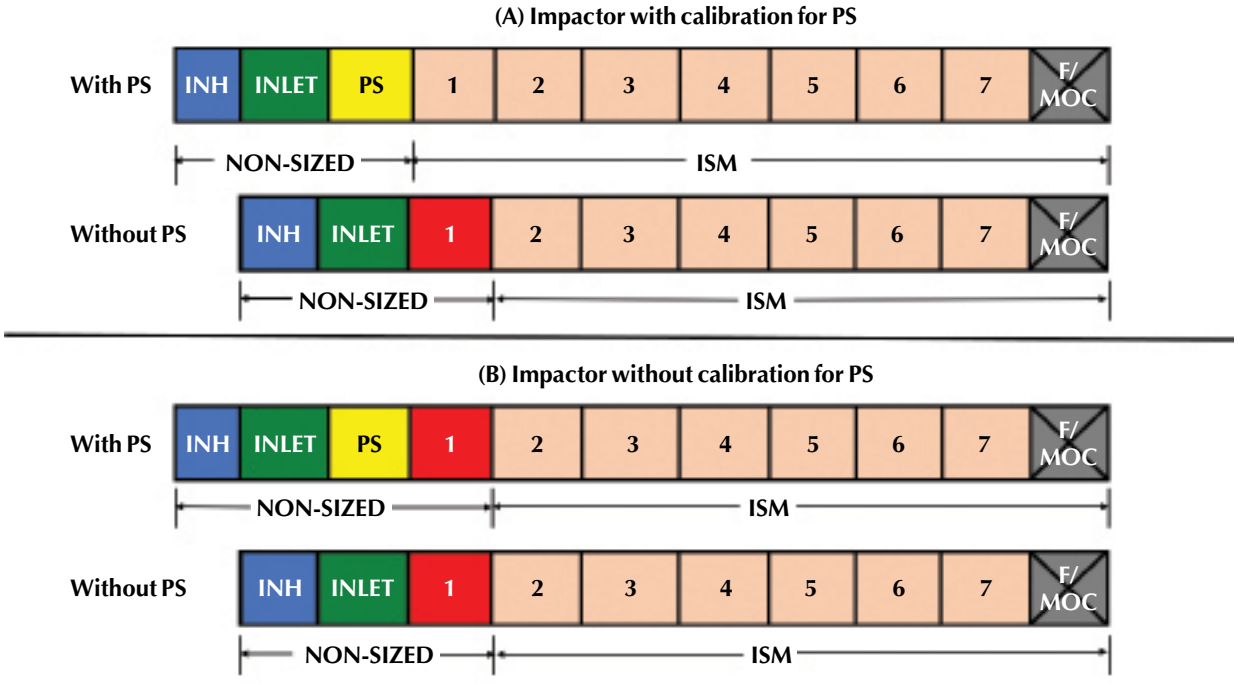
In cases where the mass of API on the initial sizing stage is excluded from the calculation of ISM, it follows that the mass recovered from that stage is assigned to the non-sized mass fraction in order to maintain the integrity of the mass balance for the measurement.

Misconception 2: Geometric standard deviation (GSD) is always suitable as the measure of spread of a size distribution.

GSD is frequently encountered as a means of reporting the spread of a size distribution [1], along with the associated measure of central tendency, typically the volume median diameter (VMD) obtained from LD-derived data or MMAD from either cascade impaction or TOF-analyzer based measurements. GSD is related to the ratio of the diameters corresponding

Figure 1

Non-sized and sized mass fractions of an idealized OIP aerosol sampled by a cascade impactor train, including USP/PhEur inlet and pre-separator, showing the importance of the calibration of the PS in determining how ISM is calculated.



to the 15.9 ($d_{15.9}$) and 84.1 ($d_{84.1}$) percentiles of the total mass associated with the distribution, whether or not scaled in terms of aerodynamic or physical size, in accordance with:

$$GSD = \sqrt{\frac{d_{84.1}}{d_{15.9}}} \quad [1]$$

For a perfectly monodisperse aerosol, GSD = 1.0, but inhaler-generated aerosols typically have GSD values that lie between 1.8 and 2.5 [14, 15].

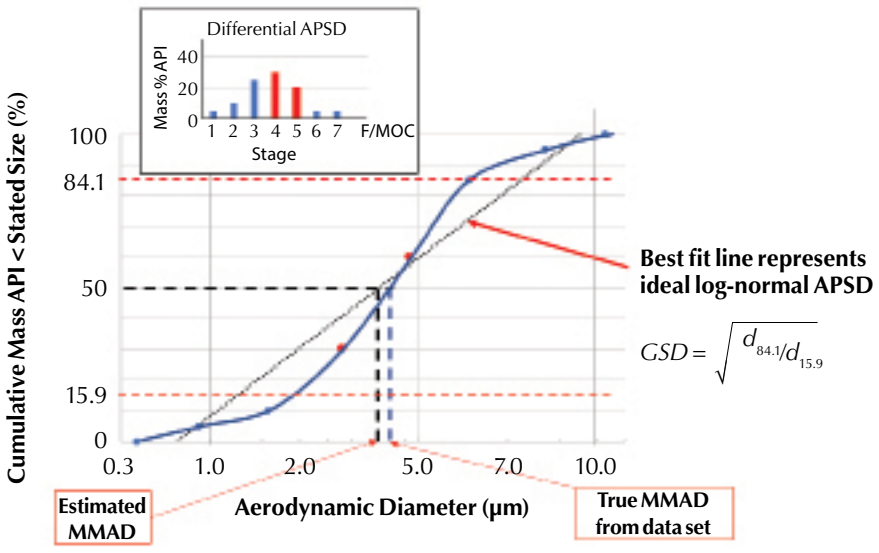
Although many OINDP aerosols/droplets, especially the non-ballistic fraction emitted by a pMDI that is captured by the cascade impactor, have been found close to being unimodal and log-normal in terms of their underlying size distribution [16], there is no fundamental physical reason why this outcome should always be the case. For example, bimodal distributions have been observed with droplets produced from aqueous nasal spray pumps and measured by laser diffractometry [17]. In another example, unimodal APSDs either having a shoulder or a fully bi-modal distribution have been reported with a DPI-generated aerosol, again sized by LD [18]. Laser diffractometry measurements are typically made over a much wider size range (ca. 0.5 to 500 µm physical diameter) than the range of operation of cascade impactors (ca. 0.1 to 15 µm aerodynamic diameter). It follows that the risk of encountering bimodality or severe variations from unimodal behavior with measurements made by laser diffractometry, is correspondingly increased.

The issue can be circumvented by constructing the cumulative mass-weighted form of the size distribution, which is non-parametric in nature, and therefore makes no assumptions about the distribution shape [19]. If the cumulative size distribution plotted with the size axis scaled logarithmically to the base 10 is linear, then GSD can be calculated by applying equation [1], as the underlying distribution is indeed unimodal and log-normal. Figure 2 illustrates typical data generated from an OIP, sampled using an NGI. The data points of the cumulative curve deviate somewhat from linearity, however the APSD is unimodal. MMAD can therefore be determined as the size corresponding to the 50% percentile of the distribution. However, reporting GSD as the measure of spread would be questionable. This figure illustrates a further complication, in the likely case in which the 50th percentile does not coincide with a cut-point size of a particular stage. Here, linear interpolation between the mass percentiles associated with the two stages on either side and nearest to this percentile (see the red data points on the cumulative curve) has been shown to provide similar accuracy to the use of curve-fitting algorithms encompassing the data from the entire distribution [20]. This outcome arises partly because, by definition, the bulk of the mass has been collected on stages closest to the 50th mass percentile (see the red histogram bars of the inset differential APSD, expressed in terms of stage number rather than a size range).

A similar approach can be used to estimate VMD from laser diffractometry data, however, this value is normally provided by the software used to generate the data report. Whichever apparatus has been used to make the measurement, if the cumulative size distribution visibly deviates from linearity, it is more appropriate to report the measure of spread in terms of the span. Span is typically calculated as the differ-

Figure 2

Representative cascade impactor-derived data set plotted in terms of cumulative mass % smaller than the stated size against aerodynamic diameter, scaled logarithmically to the base 10 to check visually if APSD is log-normal and unimodal, also showing linear interpolation method to estimate MMAD.



ence between the sizes corresponding to the 90th and 10th percentiles, in this case normalized by dividing by the volume median diameter (the size corresponding to the 50th percentile). In the case where distinct bimodality is present, the option also exists to analyze each mode separately.

Misconception 3: The underlying size distribution can be described by a single size-based metric.

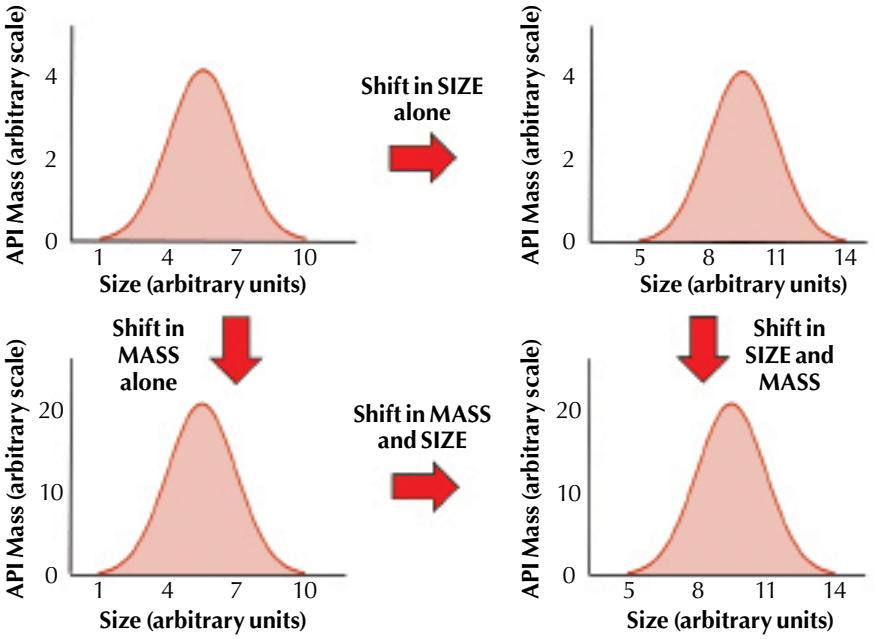
A single size-based metric, such as fine particle mass (FPM), typically taken to be less than a given size such as 5 µm aerodynamic diameter, is occasionally reported as being descriptive of the underlying size distribution of aerosols sampled from OIPs. Although $FPM_{< 5.0\,\mu m}$ is recognized by the European Medicines Agency (EMA) as a parameter to consider controlling for these products [1], there is also the understanding that batch acceptance criteria should assure a consistent APSD in terms of the (mass) percentage of total particles in discrete size ranges, representative of the median, and defined upper and/or lower particle size limits. The requirements of the United States Food and Drug Administration (FDA) are more specific in this respect [2]. Although batch acceptance criteria are suggested for groupings of consecutive impactor stages rather than proposing a criterion for each individual stage, the advice is given that, in most cases, three or four groupings should be sufficient to characterize the APSD adequately. However, stage groupings and stage-by-stage comparisons are inadvisable when the impactor has been used to acquire data at different flow rates.

A common misconception is the assumption that a measure of $FPM_{< 5.0\,\mu m}$ obtained by cascade impactor is equivalent to mass deposition within a specific region of the lung. For a given APSD, $FPM_{< 5.0\,\mu m}$ and lung deposition of inhaled API(s) are dependent on the collection efficiency of the measurement apparatus and oropharyngeal region respectively, which in turn are dependent on both flow rate and transit time. The time-course of these two variables is quite different; the impactor operates at a constant flow rate, whereas patients inhale with a continuously varying flow profile [19]. Furthermore, the size-selectivity of the particle deposition processes at both the oropharyngeal (extra-thoracic) region and distally in the lower respiratory tract is gentler than the sharp size discrimination of impactor stages [19]. Consequently, the assumption that a measured value of FPM is equivalent to lung deposition is fundamentally flawed, and measures of fine particle fraction overestimate the true lung deposition fraction obtained by *in vivo* techniques [21].

Apart from grouping impactor stage data, there are several more discriminating ways in which the large amount of information contained in a particle size distribution can be reduced in order to provide a comprehensive set of metrics that are capable of tracking all the potential changes that can take place from one batch of an OIP to another. It is essential, however, to appreciate that changes to the two fundamental properties, which are the mass of sampled API and the corresponding sizes of the particles, are tracked simultaneously (Figure 3). The most fundamental measures, ISM, together with a measure of central tendency for the size distribution (MMAD) are said

Figure 3

Model unimodal particle size distribution showing the potential for independent shifts in API mass and size; two orthogonal metrics are required to track changes in both directions.



to be orthogonal, because they meet this criterion. The addition of mass-weighted sub-fractions representing clinically relevant size ranges covering extra-fine, fine and coarse particles, provides *supporting* information, provided they are each related to ISM.

Although not yet accepted as "mainstream," the Effective Data Analysis (EDA) metrics, the sum of the small particle mass (SPM) and large particle mass (LPM) of the underlying APSD, together with the ratio metric, LPM/SPM, are also capable of meeting the above criterion [22]. This outcome arises because the sum of SPM and LPM equates to ISM, and the ratio metric is sensitive to movements along the size axis, provided the boundary between small and large particle sizes is chosen to be within 0.3 and 3 times the MMAD [23]. It is self-evident from the foregoing explanation that EDA is equally applicable to data reduction of particle size distributions sampled from all types of OINDPs, including measurements made either by TOF analyzer or by LD.

Misconception 4. Count/number-weighted size distribution data are a surrogate for volume/mass-based data.

The various particle size measurement options available to characterize OINDPs do not all determine particle size to the same weighting. Mass-based measurements are most fundamental for OINDP assessments because they equate directly to the dose of API likely to be deposited at the target site in the human respiratory tract [5]. Laser diffractometry systems determine volume-weighted size distribution data but are commonly treated as mass-based, especially as most OINDPs, with the exception of dry powder inhalers, deliver their formulations as aqueous droplets (density close to that of water, 1.0×10^3 kg.m^{-3}). This assumption also enables the size axis to be treated as being scaled in terms of aerodynamic diameter, as the droplets are spherical (dynamic shape factor of unity) and have the reference (water) density.

Some size-measuring instruments, notably TOF-based analyzers such as the Aerodynamic Particle Sizer Spectrometer (APS, TSI Inc., St. Paul, MN, US) can transform the number-weighted distribution to a mass-weighted basis, using the relationship for each size channel of the differential size distribution [24]:

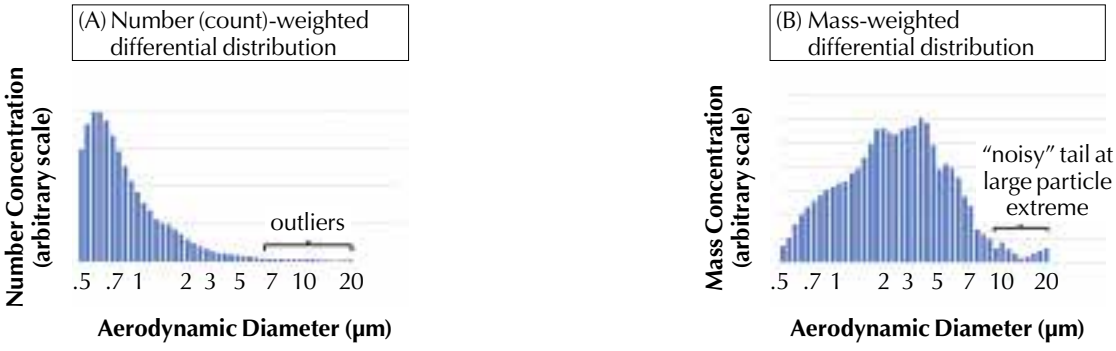
$$dM_{D_{ae}} = \left[\left(dN_{D_{ae}} \frac{\pi}{6} D_{ae}^3 \right) \left(\frac{\rho_0 C_{ae} \chi}{C_{ve}} \right)^{3/2} \frac{1}{(\rho_p)^{1/2}} \right] \quad [2]$$

in which N_{Dae} represents the number of particles collected in each size channel of the analyzer with representative (mid-point) aerodynamic diameter (D_{ae}), M_{Dae} is the corresponding mass of the particles in the particular size channel under consideration, C_{ae} and C_{ve} are the Cunningham slip correction factors associated with the aerodynamic and corresponding volume equivalent diameter (both are close to unity within the size range of interest when size-analyzing OIP aerosols), ρ_p and ρ_0 are the particle density and water (reference) density respectively, and χ is the dynamic shape factor (unity for spherical particles/droplets).

This transformation is effective when the underlying size distribution is both narrow in size range and well-defined and ideally without outlier oversize particles. In practice, however, both conditions may not be met with inhaler-generated aerosols, particularly when the aerosol is available for such a short time, that the true APSD cannot be accurately or representatively sampled. Under these circumstances, a few outliers can have a disproportional influence on the mass-weighted APSD because of the magnification introduced by the D_{ae}^3 term in equation [2] (Figure 4). Even though the user of these instruments can utilize the instrument software to transform the measured size distribution as described, it is important to understand that a fundamental misconception applies that the distributed parameter measured by the instrument can be transformed to a distribution of some other parameter *while maintaining the accuracy intrinsic to the measured data*. There are logical assumptions that can be made (such as particle sphericity) to assist the transformation process. However, these assumptions always detract from the intrinsic accuracy available

Figure 4

The effect of outlier large particles on the transformation of a number (count)-weighted to a mass-weighted APSD, from a TOF-analyzer-based determination.



in a direct measure of the mass-weighted APSD. The cascade impactor remains the preferred technique for characterizing OIPs [1, 2] mainly for this reason.

In Summary: Options for analyzing OINDP size distribution data

Several years ago, the United States Pharmacopeial Convention, through its General Chapters-Dosage Forms (GC-DF) committee recognized the need to update the basic information about the analysis of size distribution data that had been provided in General Chapter (GC) <601>. As a result, the Aerosols sub-committee of the GC-DF committee continues to collaborate with members of the Statistics committee to develop a new informational GC <1604> that focuses on ways to interpret APSDs from OIPs. A Stimulus to Revision article from this group has provided a roadmap to make this process unambiguous [25]. Of necessity, the process chart focused on data from cascade impactors, given the dominance of that methodology in mandatory GC <601>. That roadmap is repeated here, with a minor change to indicate when the use of GSD is appropriate (see Figure 5). An initial version of GC <1604> has since been published for public comment and revised, and the updated version was published in *Pharm. Forum* in September 2020 [26]. The inclusion of impactor stage grouping as part of the analysis was included to harmonize with the draft FDA guidance on the topic in relation to pMDI and DPI products [2]. The underlying principles are also applicable to TOF-based and LD-based size distribution data, disregarding stage grouping, provided an additional check is made that the size distribution is unimodal.

Dedication

We dedicate this article to the memory of the late Dr. Dennis Sandell (S5 Consulting, Blentarp, Sweden), who was a major contributor to the draft USP Chapter <1604>, as well as being a mentor to all of us on statistical methods in association with inhaler performance assessment.

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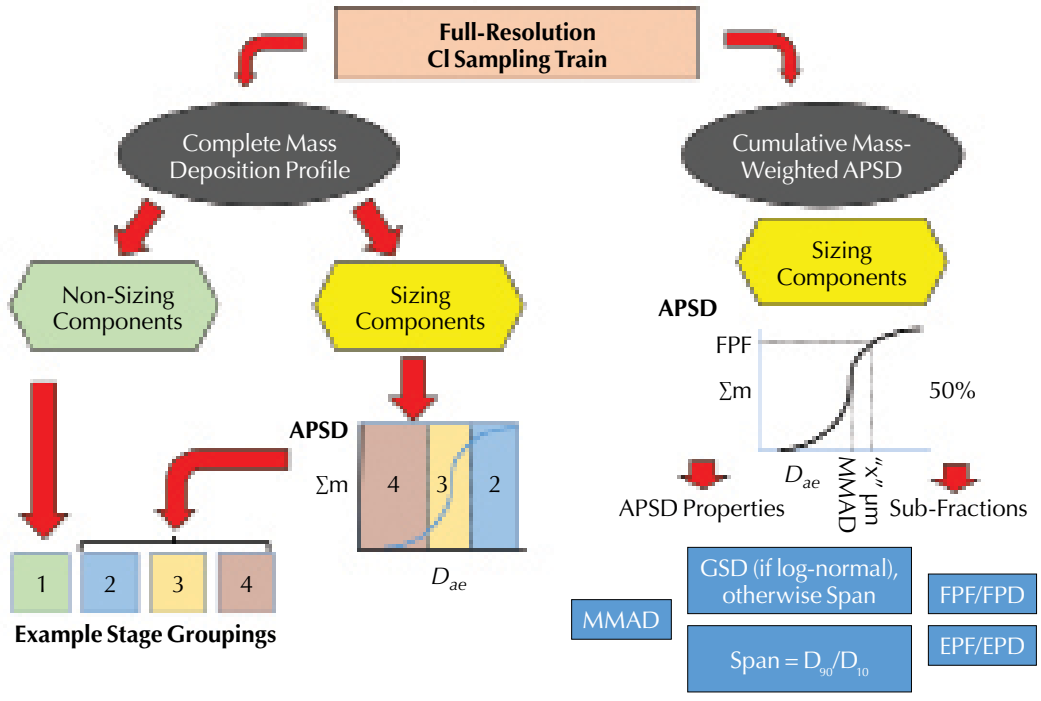
Figure 5

Roadmap for analyzing OIP APSD data determined by cascade impaction; this strategy was proposed for Draft USP Chapter <1604>, currently in revision following its first public review. Used by permission of the United States Pharmacopeial Convention (USP). This figure has been had been "adapted" from the original graphic (Figure 7) that appeared in the article titled, "An overview of general chapter development for oral and nasal drug products (OINDPs) at the United States Pharmacopeia (USP): Part 2-Informative chapters <1601>, <1602>, <1603> and<1604>," published in the December 2018 issue of *Inhalation*.



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