Weighing in on NGI gravimetric cups

A valuable tool to consider

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The NGI and its role in inhaled drug delivery

Impaction-based methods for determining the aerodynamic particle size distributions (APSDs) of aerosolized drug products intended for oral and nasal inhalation are preferred by regulatory authorities because, unlike non impaction-based approaches, impaction-based techniques such as the cascade impactor can be used to directly classify the APSD in terms of the drug substance content of the sampled drug product. One such impactor, the Next Generation Impactor (NGI), is commonly used in the pharmaceutical industry to measure APSD over a calibrated flow rate range of 15 to 100 L/min [1-3]. This article has been written to raise awareness of current and evolving approaches to APSD measurement using an NGI.

The method for determining APSD using an NGI has been adopted by the United States Pharmacopeia (USP) for various medical inhalers. Specifically, USP <601> designates the NGI as Apparatus 5 for dry powder inhalers (DPIs) with a flow rate range of 30-100 L/min and Apparatus 6 for pressurized metered dose inhalers (pMDIs) at a flow rate of 30 L/min, and USP <1601> directs use of the NGI for nebulizers at a flow rate of 15 L/min [4, 5]. The current USP method details approaches to APSD determination that involve a chemical assessment of the sample retained on each NGI cup using a drug-specific content assay. In this assessment, the sample is extracted using a compatible solvent or diluent and the drug mass in the sample is quantified using a suitable chemical or biological method. A potential drawback of this approach is that the sponsor is required to develop a sensitive, discriminating and reproducible content or potency assay at a relatively early stage of product development. High performance liquid chromatography (HPLC)-based or ultra-performance liquid chromatography (UPLC)-based content assays are readily available for most small molecules and the variability associated with these assays is typically low enough to enable differentiation between NGI cups. However, there are some cases wherein a small molecule content assay may be difficult to develop or require a complex detection method with associated high variability due to lack of a chromophore (e.g., amikacin, tobramycin and lung surfactant). In the case of biological products (e.g., attenuated adenoviruses, bacteriophages), the variability of commonly applied purity or potency assays may be so large that differentiation between the amounts of drug substance captured on the various stages becomes impossible. Thus, in such cases of high assay variability the drug-specific approach is not well-suited to impactor-based APSD determination because it may lead to an inaccurate description of the APSD and a simpler gravimetric approach with lower variability is therefore warranted.

Figure 1

NGI gravimetric inserts (small cup) with a filter substrate.

In addition to the standard configuration used for drug-specific APSD determination, the NGI vendor also provides an optional add-on accessory of stainless steel NGI™ gravimetric insert cups (Figure 1; NGI Model 170, MSP, a division of TSI, Shoreview, Minnesota). These cups are available with 55 mm filter substrates for use in NGI stages 1 through 7 and a 75 mm glass fiber filter for the micro-orifice collector (MOC). The gravimetric insert cups have been utilized in the assessment of dry powder inhaler products (DPIs) during early stages of product development [6-8]. The filters used with these cups can be easily weighed before and after an experiment to determine the stage-by-stage particle mass and corresponding APSD of a dry powder formulation. Similarly, it is possible to determine, gravimetrically, the APSDs of aerosolized liquids and suspensions using suitably designed gravimetric insert cups. This article presents several scenarios describing the use of NGI gravimetric cups/inserts during drug development and provides a detailed exploration of their utility in the measurement of APSDs for liquids and dry powders.

The NGI gravimetric method to mitigate particle bounce for dry powder formulations

A few investigators have reported the use of NGI gravimetric insert cups to measure APSDs of dry powder formulations. As part of an *in vitro* aerosol performance study intended to satisfy USP <601>, Acosta, et al. (2021) used the insert cups to sample a spray-dried, engineered powder dispersed from three different DPIs at 60 L/min (HandiHaler®, Boehringer Ingelheim, Ingelheim am Rhein, Germany; NeoHaler®, Novartis, Basel, Switzerland; and Aerolizer®, Novartis, Basel, Switzerland) [6]. Similarly, others have also utilized NGI gravimetric insert cups to measure the APSDs of spray-dried cyclosporine A and antibiotic microparticles and nanoparticles [7, 8]. The authors claimed that the gravimetric insert cups were used to minimize particle bounce and powder re-entrainment during NGI measurement.

The successful capture of a particle in an NGI cup, whether drug-specific or gravimetric, depends on adhesion of the particle to the collection surface in the cup. If adhesion does not occur, the particle may bounce back into the air stream and not deposit in the cup. This particle bounce behavior has been commonly observed when using a standard NGI cup to measure APSDs of dry powder formulations. Particle bounce occurs when the rebound kinetic energy of a particle is greater than its adhesion energy and is influenced by both the size and composition of the particle. Larger particles and those composed of harder materials are more likely to bounce when the particle velocity is high.

Particle bounce detracts from the ability to accurately determine APSD through reduced collection efficiency, increased wall losses in the downstream cups and shifted mass distribution among stages. Most of the large particles are lost on the interstage walls while small particles are often found not only on the interstage walls but also in the subsequent cups or final filter.

When a moving particle contacts a surface, part of its kinetic energy dissipates, which enables the particle to adhere to the surface (KE_a). In parallel, another portion of its kinetic energy converts to the kinetic energy of rebound (KE_b). In cases where KE_b exceeds KE_a, the particle is likely to bounce. Cheng and Yeh (1979) [9] defined the critical velocity for bounce to occur as a function of particle aerodynamic diameter and composition based on the semi-empirical relationship shown in Equation 1:

$$V_c = \sqrt{\frac{\rho}{\rho_0}\frac{\beta}{d_a}} \quad (1)$$

where V_c = critical velocity, ρ and ρ_0 are the particle density and unit density, respectively, and d_a = aerodynamic diameter. The parameter β depends on particle composition and geometry and can range from 2.5 to 9.2. A value of 5 is typically chosen for β when this relationship is used to describe particle bounce in impactors. In order to estimate the appropriate particle size and velocity to minimize bounce in an impactor, Equation 1 can be simplified for a unit density particle to the following (Equation 2):

$$V_c d_a \leq 5.10^{-6}\ m^2/s \quad (2)$$

Similarly, Dahneke (1971) [10] showed that the required KE_b for bounce to occur when a particle hits a surface depends on the particle velocity as well as the compositions of both the particle and the surface (Equation 3):

$$KE_b = \frac{d_p A(1-e^2)}{2xe^2} \quad (3)$$

where x is the separation distance, d_p is the mean particle diameter, A is Hamaker's constant and e is the coefficient of restitution. Hamaker's constant incorporates particle-substrate Van der Waals forces into the kinetic energy estimation and the coefficient of restitution relates the particle velocity after collision to the particle velocity before collision.

The adhesion force relies on the properties of the particle (size and composition), the characteristics of the collection substrate (material, roughness and/or surface treatment) and the particle velocity. The adhesion force is typically proportional to the diameter of the particle whereas the bounce force is proportional to higher degrees of particle diameter. Therefore, it would seem that a smaller particle (< 10 µm) would be less

likely to bounce than a larger particle. This phenomenon is described in Figure 2.

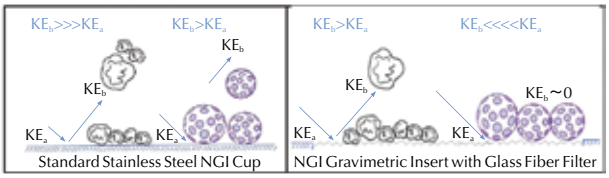
Additionally, as particles accumulate on the collection surface above a single layer, they may undergo re-entrainment either as individual particles or agglomerates. Both particle bounce and re-entrainment of particle agglomerates have been previously observed during NGI testing of dry powder formulations. It is known that such re-entrainment may interfere with the validity and accuracy of APSD results, as it tends to bias the APSD data toward finer sizes due to particles depositing preferentially in smaller size-cutoff cups. This then leads to an overestimation of the smaller respirable particle fraction. Khalili, et al. (2018) [11] studied the impact of different coating materials on particle bounce in NGI cups. It has also been shown that coating of the standard NGI cups (e.g., with Tween 80 in ethanol, Brij 35 in glycerin, Span 85/silicone oil in hexane) may help ameliorate particle bounce. However, the use of coating materials may complicate or interfere with the chemical assays of the active ingredients in the inhaled products. Careful selection of coating materials is thus needed to ensure they are compatible with the relevant drug-specific assays, which can require additional resources and result in longer method development timelines.

The surface of a standard stainless steel NGI cup is roughened, purposely, to allow coating solutions to spread easily. In comparison, an NGI gravimetric insert can be made of any suitable fibrous material with a surface that is porous as well as less rigid and naturally rougher than that of stainless steel, which serves to reduce the bounce force. Poor adhesion of particles to a standard NGI cup may therefore be improved by switching to an NGI gravimetric insert because the NGI gravimetric insert facilitates adhesion of particles when they impact the surface. Chang, et al. (1999) [12] investigated the possibility of achieving high particle collection efficiency on uncoated substrate surfaces by varying the collection material (i.e., glass fiber filter, nucleopore filter and Teflon tape), and found that the glass fiber filter displayed superior characteristics in terms of minimizing particle bounce. The improved collection efficiency was likely due to the porous, mesh-like microstructure of the glass fiber filter. A particle impacting such a surface is hypothesized to partially penetrate the mesh, thereby dissipating its kinetic energy and decreasing its tendency to bounce.

In addition to the requirement for APSD measurement, USP <601> calls for a mass balance (MB) to be performed to account for all drug emitted from an inhaler. In comparison to chemical analysis, it would be difficult to gravimetrically quantify deposits captured in the USP Induction Port and the interstage passageways of the NGI, due to the large masses of these system components relative to the amount of deposited powder. However, if the cumulative mass of drug collected in all other components is between 85% and 115% of the nominal, the results should be considered valid according to USP <601>.

Figure 2

Non porous (black) and porous (purple) particles impacted on a standard NGI stainless steel cup and an NGI gravimetric insert with glass fiber filter. KE_b is dominant when non porous particles land on a hard surface while KE_a is dominant when porous particles land on a fibrous surface. Note that this schematic only applies to a single layer of deposited particles.



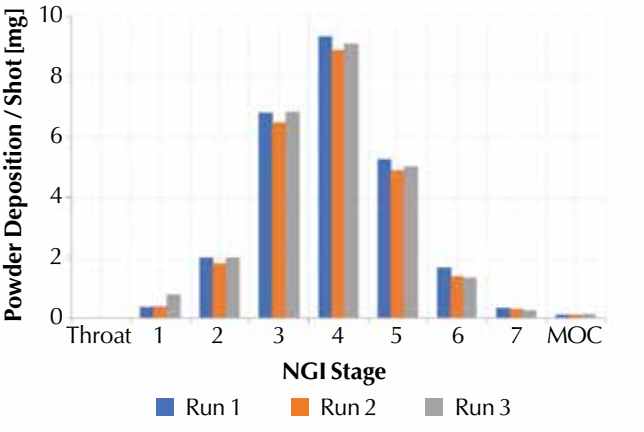
An example for dry powder formulations

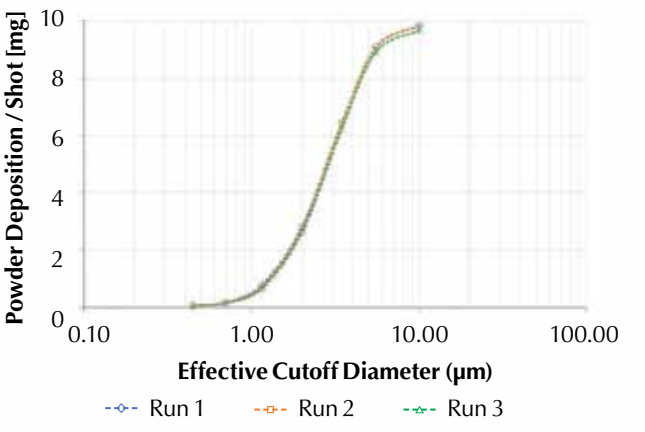
Here we present an example wherein NGI gravimetric cups were used to obtain APSD data on Tobramycin (PulmoSphere™, Viatris, Canonsburg, PA, US) particles manufactured by spray drying a perfluorooctyl bromide (PFOB)-in-water emulsion. In this emulsion, the drug is dissolved in the continuous phase and the PFOB emulsion acts as the dispersed phase. PFOB is an immiscible liquid with a very low vapor pressure that evaporates much more slowly than the aqueous phase of the emulsion. The PFOB droplets retard particle shrinkage during particle drying and remain until after the particle shell has solidified, which results in the highly porous PulmoSphere particle structure. These particles therefore exhibit a very low tapped density (< 0.2 g/cc) compared to particles spray-dried from simple solutions or suspensions. Because this powder is spray-dried from a uniform feedstock, its composition is homogeneous and therefore the distribution of drug closely follows the distribution of powder mass. A 40 mg capsule fill mass was evaluated for APSD using a high flow resistance device (RS01 – R:0.16; Plastiape S.p.A., Osnago, Italy). NGI gravimetric cups with dry filter substrates were used to sample aerosolized tobramycin at 40 L/min. Previously, this powder had been known to exhibit particle resuspension issues during testing with an NGI standard cup that required resolution using coating materials (data not shown). As shown in Figure 3, the NGI gravimetric stage distributions were reproducible and did not display noticeable accumulation in the higher-number stages, which implies the lack of obvious particle bounce.

Although in many cases particle bounce and re-entrainment can be sufficiently overcome by the use of the NGI gravimetric insert cups with less rigid collection surfaces, there are situations in which this may not

Figure 3

NGI gravimetric stage distributions with PulmoSphere™ powders (left) and their cumulative percent distributions (right).





be sufficient to dissipate the rebound kinetic energy. In such instances, the insert filter for each cup can be hydrated so that particles will more readily adhere to its wet surface. The presence of a liquid layer on the collection surface is known to significantly improve the likelihood of adhesion at impact, due to the action of the capillary force between the particle and the surface. This phenomenon is exemplified using an albuterol (PulmoSol™) powder, which was spray-dried from a homogeneous aqueous solution of albuterol and other water-soluble matrix-forming excipients (i.e., leucine) under conditions that resulted in particles with a classic collapsed "raisin-like" morphology due to the presence of leucine as a shell former. The albuterol powder was filled into capsules at a fill mass of 45 mg, dispersed using a high flow resistance DPI (RS01-R:0.16; Plastiape) and sampled at 40 L/min using an NGI equipped with NGI gravimetric cups with dry filter substrates (Figure 4). The test was then repeated using wet filter substrates. A comparison of the two APSD data sets clearly shows that particle bounce can be mitigated by wetting insert filters to collect materials with greater KE_b such as PulmoSol particles.

Similarity of the gravimetric APSD results obtained using NGI gravimetric insert cups to drug-specific APSD data analyzed using a UV method was demonstrated for the above spray-dried albuterol powder. Both data sets were obtained from the same samples, collected using NGI gravimetric insert cups with dry filters. As shown in Figure 5, both methods displayed analogous size distributions thereby verifying applicability of the gravimetric method for measurement of engineered powders.

In a separate study, Dunbar, et al. (2005) [13] showed how bounce effects could be minimized by saturating 20 µm pore glass fiber filters with water and placing these filters on inverted impaction plates in an Andersen Cascade Impactor (ACI). In this study, good agreement was obtained between the ACI APSD and the APSD measured using a Multistage Liquid Impinger (MSLI); i.e., mono-modal with no statisti-

Figure 4

NGI gravimetric stage distributions with dry and wet substrates (PulmoSol)—45 mg fill mass.

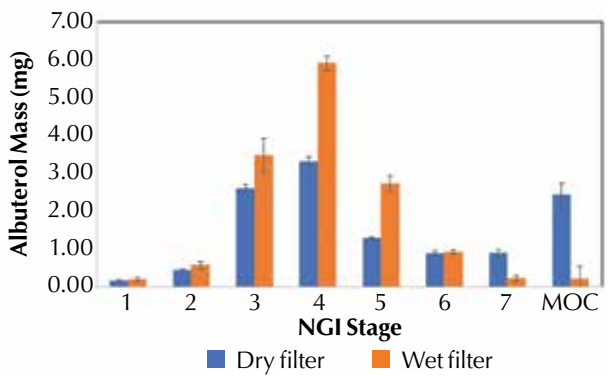
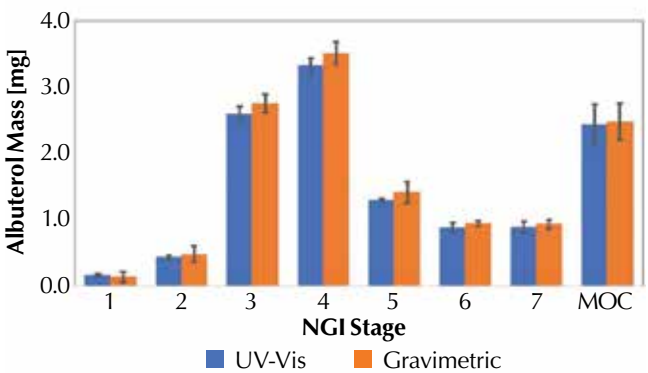


Figure 5

Comparing NGI gravimetric stage distributions by ultraviolet (UV) and gravimetric assays.



cally significant differences in MMAD and σ_g. This study demonstrated how selection of the impaction substrate material and solvent must be evaluated with the specific drug product and analytical methods to minimize bounce effects and obtain an accurate measure of the APSD.

NGI gravimetric applications for liquid formulations

Published literature on the use of NGI gravimetric inserts has previously been limited to their application for measurement of APSDs of dry powder formulations. In contrast, data have not been provided in the literature that illustrate the use of NGI gravimetric inserts for measuring APSDs of nebulized solutions or homogenous suspensions. Since particle bounce and resuspension are not likely to occur with liquid-based formulations, an NGI gravimetric approach would be especially useful for APSD measurement of these formulations, as they typically exhibit uniform drug content across the entire particle size distribution. As a result, an APSD obtained using NGI gravimetric inserts should accurately reflect the drug-specific APSD for a given formulation.

Measurement of APSDs for liquid aerosols using standard NGI stainless steel cups is performed at a flow rate of 15 L/min for a predetermined sampling time to avoid overloading the cups. Such overloading can result in the formation of droplets that streak across the cup surface and change the gap between the stage and the NGI cup. In contrast, when an NGI gravimetric insert is used, the fibrous filter material (e.g., glass fiber) may obviate overloading issues due to its ability to absorb liquid droplets that land on its surface. As a result, the NGI gravimetric inserts enable the use of longer sampling times, which can help reduce both assay and weighing variability due to collection of larger samples without compromising APSD measurement. USP <1601> recognizes the difficulty of comparing the total dose collected during nebulizer testing to the total mass collected in a cascade impactor test and suggests that evaporation is an important reason for this difficulty. Consequently, USP <1601> recommends that recovery experiments be performed as part of method development and validation, and that evaporation be controlled by cooling the impactor.

An example for liquid formulations

In this section we present APSD data, obtained using NGI gravimetric inserts, for a uniform pulmonary drug product suspension aerosolized using a jet nebulizer. In this evaluation, a 6 mL nebulizer charge volume was tested for different sampling times (i.e., 2 min and 12 min). Each NGI gravimetric insert filter could then be evaluated chemically or gravimetrically (post drying). The APSD data obtained for this drug product using NGI gravimetric insert cups are shown in Table 1 and Figure 6. It is useful to note that the mass on each NGI cup increased proportionally with increasing nebulizer sampling time while the APSDs remained the same for the three sampling times studied. These data demonstrate that the NGI gravimetric inserts can be used to measure larger sample quantities, including potentially the entire nebulizer fill volume.

Use of NGI gravimetric inserts for APSD measurement may also be considered as an alternative to a laser diffraction (non impaction-based) technique during preclinical and early clinical development of nebulized solutions and homogeneous suspensions. A laser diffraction approach is allowed by USP <1601>, although it must eventually be validated against an impaction-based sizing method. However, as mentioned previously, this requirement may be very diffi-

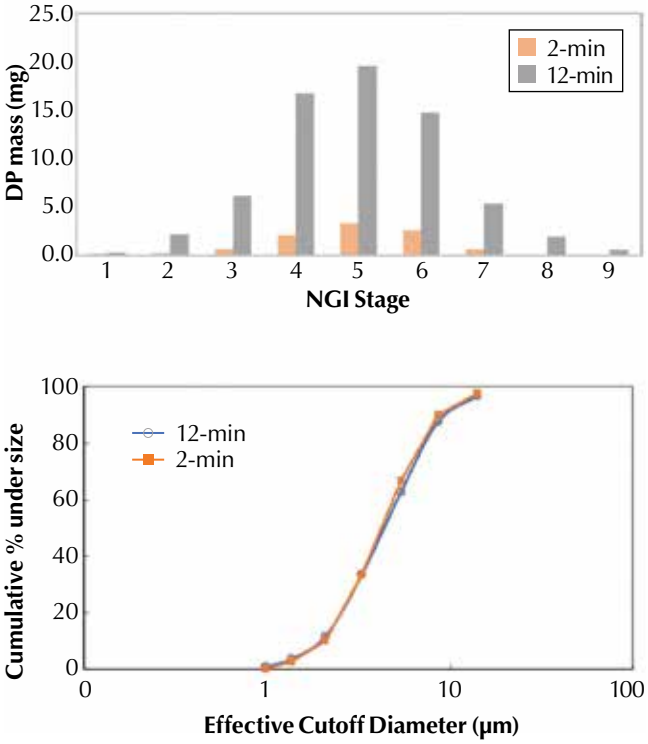
Table 1

APSD of drug product (a homogenous suspension) based on NGI gravimetric insert.

Sampling Time [min]	MMAD [µm]	GSD	%FPF$_{<5.0\,\mu m}$	%FPF$_{<3.3\,\mu m}$
2	4.1	1.7	64	34
12	4.4	1.7	58	33

Figure 6

NGI gravimetric percent stage distributions with homogenous suspension (top) and their cumulative percent distributions (bottom) at two sampling times.



cult to achieve for products with complex assays and/or products having high assay variability. In addition, during early development, a drug-specific assay needed to enable APSD determination using an NGI with standard cups may not yet be available. In such cases, use of the NGI gravimetric method instead of a laser diffraction method for APSD determination of a nebulized product could represent a compromise approach, as it would likely be more acceptable to regulators over a non impaction- based method due to its direct mass quantification.

The NGI gravimetric method as a valuable tool

We have provided a rationale for and examples demonstrating the applicability of NGI gravimetric insert cups for measurement of APSDs of homogeneous liquid suspension and dry particle aerosols. The NGI gravimetric method can play a role as an alternative or complement to drug-specific NGI or non impaction-based methods such as laser diffraction, especially as a means to avoid drug-specific assay variability or to satisfy regulatory expectations for impaction-based APSD approaches. The NGI gravimetric approach may also help to address aerosol characterization needs in phase-appropriate product development strategies. Researchers and product developers are encouraged to consider the NGI gravimetric method as a valuable tool in liquid and dry powder aerosol measurement.

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