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Comparison of Cascade Impaction and Laser Diffraction for Particle Size Distribution Measurements

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ABSTRACT

The Andersen cascade impactor (ACI) and laser diffraction (LD) can be correlated at ambient temperature for aqueous drug formulations atomized by Soft Mist™ inhalers. A comparison of the two particle size determination methods at different conditions (flow rate, relative humidity) was performed. Under well-defined conditions, the faster LD can substitute the time-consuming ACI at least for routine tests. The measurements were performed with three different drug formulations. The aerosol was generated by Soft Mist™ inhalers, and the droplet distributions were measured simultaneously using a laser diffraction analyzer together with the eight-stage Andersen cascade impactor. The simultaneous measurements ensure that aerosol and air conditions are identical for both LD and ACI. In order to measure the scattered laser light intensity of the aerosol passing the induction port, glass windows were fitted to the induction port. The evaporation effect of the aqueous aerosols on the PSD was investigated at ambient humidity and high humidity (RH > 90%). The simultaneous determination of the droplet size distribution leads to a good correlation between the ACI and LD method only if the measurements were performed at RH of >90%. The humidity of the ambient air had the strongest influence on PSD not only for ACI, but also for LD. In our set-up, the almost saturated air prevents aqueous droplets from drying. The influence of the flow rate on LD was negligible, whereas for ACI, a flow rate dependence is expected. The advantages of LD and the demonstrated compatibility to established EP/USP methods motivate the substitution of the ACI and the use of LD for routine measurements.

Key words: soft mist inhaler, particle size distribution, impactor, laser diffraction

INTRODUCTION

In the pharmaceutical industry, the determination of particle size distributions (PSD) of atomized aerosols is important for estimating the deposition characteristic in the lungs. In practice the common principle for measuring the PSD is the impaction method as described in the USP 26.[1] The cascade impactor can be considered as a simplified model of the respiratory system of human beings. The aerosol is guided by means of an air stream at defined flow rate through the rectangular bend (model of the human throat) and the following impaction stages (modelling the particle size dependent deposition in different parts of the lung). Further information about the cascade impactor and the measurement principle can be found in a monograph series by

Boehringer Ingelheim Pharma GmbH & Co. KG, Ingelheim, Germany.

Lodge and Chan.[2] This method is well accepted by the national medical agencies due to its simplicity and robustness. The whole system is defined and can be described by only a few parameters like the flow rate of the air stream and the geometry of the impactor, for example, the number of nozzles, the jet diameters defined by the nozzle diameters of the nozzle plates, the distances of the nozzles to the impaction plates and the length of the nozzles. However, the process of aerosol analysis is time consuming and therefore not suitable for routine measurements with large batch numbers. Especially the analysis of the different mass fractions on the impaction stages is very labor intensive. Hence it is necessary to establish faster alternatives for particle size determinations based, for example, on laser diffraction (LD). A typical laser diffraction instrument and further details are given in the International Standard ISO 13320-1,[3] for example.

A laser is used to generate a monochromatic, coherent, parallel beam that illuminates the dispersed particles after expansion by the beam processing unit. In many conventional systems, the measuring zone has ambient air conditions. Enclosures are offered for light protection. The effect of ambient air interacting with the aerosol is often neglected. The incident light is scattered by the ensemble of dispersed particles. The total angular intensity distribution (I[θ]), consisting of both direct and scattered light, is then focused by a lens system onto a multi-element detector, where a discrete spatial intensity distribution (I[r]) is recorded. By means of a computer the particle size distribution can be calculated which best approximates (I[r]).

In order to introduce and establish the laser diffraction method as a tool that may replace the cascade impactor for routine measurements on pharmaceutical inhalers, the equivalence of both methods must be proven. Using continuously operating nebulizers, Clark,[4] Kwong et al.,[5] and Vecellio None et al.[6] established a good correspondence between the methods regarding the aerodynamic diameters and the geometrical standard deviations. However, only Clark[4] simultaneously measured the PSD of a non-volatile aerosol (dibutyl phthalate) with both methods.

Kwong et al.[5] used aqueous aerosols which are affected by evaporation. By laser diffraction, they investigated a free aerosol cloud. On the other hand, the standard set-up was used for the Andersen impactor measurements and unconditioned room air was entrained into the nebulizer chamber. The authors stressed the importance of humidity control during the cascade impactor measurement, and achieved this goal by cooling the cascade impactor in order to minimize evaporative losses. However, Kwong et al. did not find any evidence suggesting a significant evaporative loss of fine particles using LD.

Vecellio None et al.[6] have used a T piece sampling technique with LD in order to have the same experimental set up as cascade impactors used in European Standard EN 13544-1. The authors have demonstrated that it is important to use the same experimental set up to compare the different measurement methods; for example, when sampling at a 90-degree angle at 2 L/min air flow in accordance with EN 13544-1,[7] it was shown that LD used with T piece underestimated the MMAD of the aerosol produced by nebulization with respect to sampling at 0-degree angle at 15 L/min. The tests were performed close to standard conditions in the range of 23 ± 2°C and 40–75% RH.

As far as metering inhalers are concerned, Ziegler and Wachtel[8,9] described the first successful attempts to establish a correlation between laser diffraction and cascade impaction using aqueous aerosols generated by soft mist inhalers. Dedicated equipment is required as the soft mist inhalers generate a high particle density ($>10^6$ particles/cm^3) for a time span of $\leq$1.5 sec. The metered dose operation of the inhaler prevents the entrained air from establishing an equilibrium humidity at reduced temperature and motivates the need for assessment of individually delivered doses. A simultaneous measurement is the only way to assess one individual dose with both methods, LD and ACI, respectively. For that reason, the measurements were performed simultaneously and evaporation was accounted for by a comparison between volatile aqueous liquid formulations and non-volatile aerosols. The aqueous aerosols were generated by a soft mist inhaler. Humidified air with RH of >90% was passed through inhaler, induction port and ACI. The measurements were performed at ambient temperature (22 ± 2°C). For the simultaneous measurement of the PSD with LD and ACI the induction port (also denoted USP-throat, see USP 26[1]) was modified without changing the characteristic impactor geometry.

MATERIALS AND METHODS

Prototype Respimat® soft mist inhalers were used to generate the aqueous aerosols. The inhaler uses the mechanical energy of a loaded spring which drives a piston. A metered amount of liquid is pressed through a micro-nozzle, producing an aerosol of the desired MMD, for example, MMD of <5 μm. The investigated formulations were close to final formulations intended for market and contained different active drugs (active drug concentration c indicated) as well as excipients. They are called formulation A ($c = 0.049\%$), B ($c = 0.198\%$), and C ($c = 0.833\%$). By this choice, the concentration c of drugs ranged from $c = 0.049\%$, 0.198% to 0.833%. The density of the aqueous formulations was close to unity (1.0 g/cm^3). A single actuation of the inhaler resulted in a spray duration of 1.5 sec.

The non-volatile aerosol was generated with a Sinclair-LaMer type aerosol generator MAG-2010 (PALAS® GmbH in D-76229 Karlsruhe, Germany). This aerosol was used for testing the reliability of the laser diffraction analyser. The generator is capable to generate adjustable particle diameters between approximately 0.3 and 6 μm with a geometric standard deviation σ_g less than 1.15 and a number concentration up to 10^6 cm^{-3}. In the boiler where the aerosol material is vaporized, the temperature controls the particle diameter. The corresponding aerosol material is DEHS (Di-2-Ethylhexyl-Sebacate).

Particle size measurement

Aerosol droplet distributions were measured using the Sympatec HELOS laser diffraction analyser (Sympatec GmbH, D-38678 Clausthal-Zellerfeld, Germany) at $\lambda = 632.8$ nm (He-Ne laser) together with an Andersen Mark II 8-stage cascade impactor operated at 28.3 L/min with the corresponding cut-off points 0.4, 0.7, 1.1, 2.1, 3.3, 4.7, 5.8, and 9.0 μm. To our knowledge, the cut-off diameter of the throat is not well defined in the range from 10 to 20 μm. We assumed 10 μm as a first approximation. As another experimental restriction, particles with diameters below 1 μm are hardly detectable with the LD configuration used for the presented measurements. Therefore the comparison of the two methods is limited to one decade of particle sizes from 1 to 10 μm.

The analysis of the drug was performed in the case of formulation C with an UV/VIS scanning spectrophotometer at the wavelength $\lambda = 218$ nm and sometimes additionally at the wavelength $\lambda = 276$ nm. The detection of the other two formulations A and B was performed with standardised HPLC because of their lower drug concentrations. The amount of DEHS was determined by weight.

Particle size calibration

For the control of the reliability of the generated data the laser diffraction apparatus was tested with a reference reticle. The reference reticle consists of silicon particles of defined sizes deposited onto a glass slide. The size distribution of the reticle was measured with the laser diffraction apparatus used for the measurements and with a laser diffraction apparatus of the same type as a reference. The results were compared with the nominal values given for the reference reticle. The laser diffraction analyser including the throat (configuration with windows before the bend; Fig. 1) was additionally tested with a monodisperse aerosol. The generation process of the test aerosol is based on the Sinclair-LaMer principle by condensation of the vaporized aerosol material at nuclei. The aerosol consisted

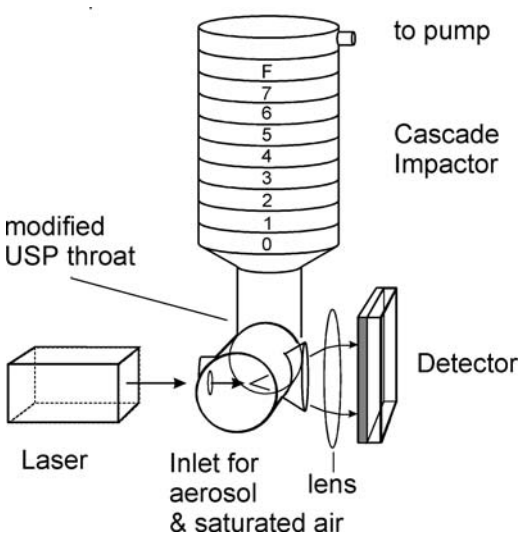

FIG. 1. Front side view of the experimental set-up for simultaneous particle size distribution measurements with the cascade impactor and the laser diffraction method. The distance from the centre of the measurement cone to the lens is 4 cm. The cascade impactor is used in a turned position for technical reasons.

of DEHS (di-2-ethylhexyl-sebacate). Three different monodisperse particle size distributions with D_{50} values between 2 and 6 μm were generated and measured simultaneously with the laser diffraction analyser and the cascade impactor.

Dedicated set-up

We decided to stay as close as possible to the induction port described by the USP 26[1] and other pharmacopeia. Therefore, the sample induction port was used and adapted to the requirements of the LD method. In addition to measurements under ambient humidity (relative humidity RH $\approx$ 30–45%) the particle size distribution was investigated under water vapor saturated air (RH $>$ 90%) conditions to study the evaporation effect of the aqueous aerosols. The air inlet vents of the inhaler or the complete inhaler device were housed and flooded with water vapor saturated air which was produced by a humidifier operating ~2°C above room temperature. Excess humidified air escaped to the surrounding. The schematic experimental set-up is shown in Figure 1.

In order to measure the scattered laser light intensity of the aerosol passing the induction port, two holes were drilled perpendicular to the air duct which were sealed with O-rings and glass windows. A three-dimensional view of the modified USP throat is presented in Figure 2. Unless stated otherwise, the laser beam crossed the aerosol exiting the inhaler before the bend of the USP throat (Fig. 2A), because the optional position "after the bend" (Fig. 2B) is expected to have a limited measurement range.

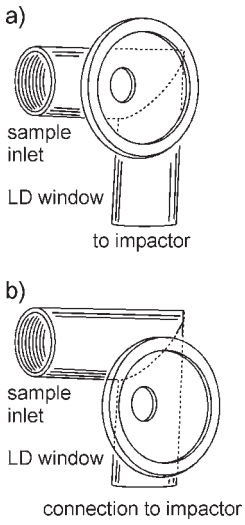

FIG. 2. Visualisation of the modified USP throat. In the direction of air flow: (**a**) Windows before the bend. (**b**) Windows after the bend. The inlet orifice for the laser beam is not visible.

This bend represents a first impaction stage for large particles, and therefore these particles can be detected neither by the laser diffraction nor by the cascade impactor. From the point of view of quality control of a spraying device, the windows positioned before the bend are preferred, because in this position all droplets can be detected by the laser system. Irrespectively of the window position it is possible with this set-up to measure the PSD with the cascade impactor and the laser diffraction method simultaneously. To ensure sufficient drug deposition on all the impactor plates to allow for UV spectrophotometric or HPLC analysis, four to eight actuations per measurement were collected. However, for the laser diffraction device one single shot would be sufficient. The laser diffraction data was analysed based on the Mie-theory which is applicable for transparent spheres. For that purpose the refraction and absorption index of the droplets must be known. The refraction index of the aqueous aerosol particles was 1.33 and the absorption was 0.0. For the DEHS particles, the refraction index was 1.45 and the absorption was 0.0. It is important to use the Mie correction to take into account the increased scattering of light from smaller droplets compared to the Fraunhofer theory.[11,12]

Data and statistical analysis

The PSD measured with laser diffraction was calculated automatically from the scattered light intensities striking the 31 detector elements. The Sympatec HELOS software used for the calculation was WINDOX version 3.3.

The basis for the calculation of the PSD measured with the cascade impactor was the total mass detected with the photometer or HPLC; that is, the total mass is the sum of all masses recovered on the different impaction stages and in the USP throat for all measurements with LD before the bend (Fig. 2A). In the alternative position "after the bend" (Fig. 2B) the mass deposited in the USP throat was excluded. The implicit assumptions for the comparison of aerodynamic diameter (d_{ae}) measured by ACI and geometric diameter (d_g) measured by LD are a constant density of the particles, for example, $\rho_p = 1$ g/cm^3 for the present aqueous formulations, and constant ho-

mogeneous concentration of drug among all droplets, the latter being required for the ACI analysis. In the Stokes regime, theory predicts $d_{ae} = (\rho_p/\rho_{ref})^* d_g$, with $\rho_{ref} = 1\ g/cm^3$. Therefore, in the present case of aqueous formulations, the diameters should be equal.

All PSD data were converted in percentage of the cumulative undersize fraction CF with relation to the cut-off diameters of the cascade impactor, for example, CF(5.8 μm) means the fraction in percentage of a particle ensemble with diameters less or equal than 5.8 μm. The PSD and the characteristic aerosol parameters D_{50}, σ_g, and fine particle fraction (FPF) (<5.8 μm) measured with the two particle size detection methods were evaluated qualitatively (visual assessment) and if appropriate quantitatively by means of a significance analysis (*t*-test, confidence intervals[13]). The correlations between the different measurements were characterized by linear regressions between the cumulated fractions of the respective size distributions.

The geometric standard deviation σ_g is given by the following:

$$\sigma_g = \left[\frac{\sum n_i (\ln d_i - \ln d_g)^2}{N-1} \right]^{1/2}$$

$$d_g = (d_1 \ldots d_N)^{1/N} \quad (1)$$

where n_i number of particles with diameter d_i; N = total number of particles; and d_g geometric particle diameter.

Under the prerequisite of a log-normal distribution (the logarithm of the particle diameters is normal distributed) the geometric standard deviation is equal to the following:

$$\sigma_g = \frac{D_{84}}{D_{50}} = \frac{D_{50}}{D_{16}} = \left[\frac{D_{84}}{D_{16}} \right]^{1/2} \quad (2)$$

Eq. 2 is used in the following for calculating σ_g. D_{50} is the median diameter, D_{16} and D_{84} are the diameters at which the cumulative size distribution reaches 16% and 84%, respectively.

RESULTS

Reliability tests

The results of the reticle measurements are shown in Table 1. In order to obtain representative results, seven measurements per laser diffraction analyser at different reticle positions were performed. The results of the test analyser, which was used for all subsequent investigations, show excellent correspondence to the reference analyser results (*t*-test, $n = 7$, $p > 0.05$). However, all nominal values are slightly but significantly (NE, *t*-test, $n = 7$, $p < 0.05$) higher than the measured ones.

Since the reticle spot diameters are quite large it is reasonable to control the reliability of the laser analyser in a size range less than 10 μm. No reticle was available in this size interval. Therefore an aerosol generator was used. The characteristic parameters of the monodisperse PSD generated by the MAG-2010 aerosol generator are presented in Table 2. Three different boiler temperatures and hence three PSD were investigated simultaneously with the laser diffraction apparatus and the cascade impactor. The cascade impactor served as the reference test method.

The D_{50} values show differences from 0.4 to 0.6 μm between the two detection methods (*t*-test, $n = 8$, $p > 0.05$). Differences of this order of magnitude are expected due to slightly different calibrations and the completely different operating principles of LD and ACI. All geometric standard deviations (*t*-test, $n = 8$, $p > 0.05$) are statistically equal.

INFLUENCE OF THE THROAT MODIFICATION ON THE PSD

The original induction port was modified and the usual position of the impactor was changed during the simultaneous measurements with

Table 1. PSD of a Reticle Measured with Two Laser Diffraction Analysers of the Same Type (Test Analyzer and Reference Analyzer)

	Test analyzer (n = 7)	*Reference analyzer* (n = 7)	*Nominal value*
D_{10} [μm] ± SD	27.49 ± 0.84	27.61 ± 0.47	30.61
D_{50} [μm] ± SD	36.85 ± 1.58	36.91 ± 1.16	39.05
D_{90} [μm] ± SD	47.03 ± 2.12	47.54 ± 2.48	49.69

The mean values of D_{10}, D_{50}, and D_{90} are compared with the nominal value. Measurements according to reticle manufacturer's instructions without throat.

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