Chapter 2 - AN *IN VITRO* COMPARISON OF THE AERODYNAMIC PROPERTIES OF TWO TYPES OF NEBULIZATION BY CASCADE IMPACTION AND LASER DIFFRACTION TECHNIQUE

A. Introduction

The pharmacological effects of an inhaled drug are related to the amount of drug reaching the lower airways and the pattern of deposition. Aerodynamic properties of emitted particles determining a preferential area of deposition are measured by two *in vitro* techniques: cascade impaction and laser diffraction. They are essential to predict pulmonary deposition of a nebulized drug and allow a comparison between devices. Fine particle fraction and mass (or volume when using lazer diffraction) median aerodynamic diameter are two major parameters to evaluate nebulizer efficacy.

The aim of these studies is to compare the aerodynamic properties of particles emitted by the intrapulmonary percussive ventilation to those obtained by a classical jet nebulizer before approaching *in vivo* measurements of pulmonary deposition and to allow an *in vitro* comparison between these properties measured either by cascade impaction or by laser diffraction.

B. Study of the aerodynamic properties of the IPV by the cascade impaction

1. Material and Method

Two nebulizers were tested: Sidestream (MedicAid, Brussels, Belgium) and IPV1 (Percusionnaire, Brussels, Belgium). Aerodynamic properties of particles were measured by an Andersen cascade impactor (1 ACFM Not-Viable Eight Stage Cascade Impactor, Graseby Andersen, Atlanta, GA). This impactor consists of a bent tube, a preseparator, 8 aluminium stages and a filter upstream of the latter. On each stage, there are 400 identical openings with a decreasing diameter along the stages.

Before nebulization, plates of the impactor were covered with one millilitre of water-soluble hydroxypropylmethylcellulose (HPMC) gel (22.5% of weight by volume of

water) to fix impacted particles. Sidestream (SST) and IPV were connected successively in a tight way to the impactor without mouthpiece. Each nebulizer was connected to an air cylinder. Pressure of the compressed air was 3.5 bars. Procedure was carried out with an entraining flow of 28.3 $L.min^{-1}$ and 60 $L.min^{-1}$ according to the standard protocol.

For both modes of nebulization, 2 ml of a fluorescent solution of 100 mL of sulforhodamine (50 μg/mL) diluted with NaCl 0.9% were nebulized during one minute. After nebulization and dissolution of the particles impacted on each plate in 100mL distilled water, fluorescence of collected solution was measured by a Perkin Elmer Spectrometer of fluorescence, model LS 50B (excitation=586 nm, emission=602 nm). This spectrometer uses a lamp with pulsated xenon as source of excitation. Final concentration of the fluorescent marker (ng/mL) was obtained by subtracting the intensity of the fluorescence of the solvent and HPMC gel, to that of the collected solution. By adding mass of the particles collected on each stage, the total emitted amount was obtained.

Estimation of the fine particle fraction was obtained following the transformation of the percentages of cumulated mass into probit values from a specific table (see addendum 1). The probit function is the inverse cumulative distribution function. Cumulated mass deposited on each stage was calculated starting with lower stage, and it was expressed in cumulated percentage (compared to total mass). The cut-off diameters of each plate were transformed into log value. Then, a linear regression was constructed from the chart for the probit value (y-axis) according to the logarithms of the cut-off diameters (x-axis). The equation of the linear regression allowed calculating FPF. Two y-values were calculated from the y-value corresponding to 5 μm and 1 μm cut-off diameters respectively. Both y-values were transformed into corresponding percentage of cumulated mass and the second one was subtracted from the first one to obtain FPF. Emitted dose expressed as percentage of the initial dose corresponded to the total mass quantified at the different plates of the impactor.

MMAD was calculated from the same equation as FPF. The cumulated mass corresponding to 50 % of the emitted dose was transformed into probit value. By

replacing the y-value from the equation of the linear regression with this probit value, the logarithm of the MMAD was obtained and then the MMAD.

2. Results

Amount of drug collected on each stages of the cascade impactor are shown in the Figure 27.

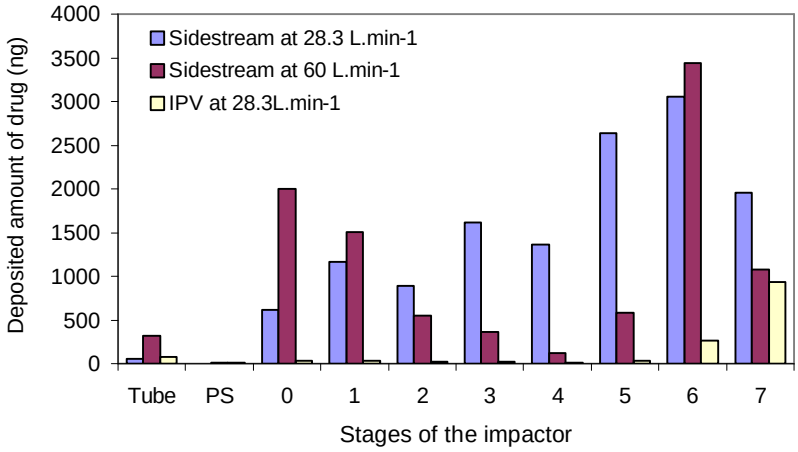

Figure 27: Deposited amount on the various stages (tube, preseparator (PS) and stages 1 to 7) of the impactor following nebulization by both techniques

FPF and MMAD are calculated by applying the equation y= ax + b as shown in Table 1.

Table 1 : Coefficient and constant of equation for each experiment

Y=ax+b	a	b	FPF (%)	MMAD (µm)	ED (%)
IPV at 28.3 L.min^{-1}	1.103	-6.206	16.2	0.2	1.45
Sidestream at 28.3 L.min^{-1}	0.348	-1.462	67.5	1.89	13.4
Sidestream at 60 L.min^{-1}	0.472	-2.279	47.3	1.21	9.98

With entraining flow at 28.3 L.min^{-1}, FPF was 67.5 % and 16.2 % for Sidestream and IPV respectively, whereas MMAD was 1.89 µm and respectively 0.2 µm. With entraining flow of 60 L.min^{-1}, only Sidestream allowed to obtain values (FPF = 47.3 % and MMAD = 1.21 µm). Emitted dose (ED) was respectively 13.4 and 9.98 % of the nominal dose for Sidestream at 28.3 L.min^{-1} and 60 L.min^{-1}. For the IPV, at 28.3 L.min^{-1}, ED was 1.45 % of the nominal dose.

3. Discussion

In summary, IPV is associated with a lower fine particle fraction, MMAD and emitted dose than SST, which should be theoretically less effective.

If *in vitro* measurements present some limitations and may overestimate results[51], they allow valid comparison of two techniques under standard conditions. The guidelines of European Respiratory Society recommend the measurement by impaction[2,157]. The technique by laser diffraction is a valid alternative[158] but, contrary to impactor which measures emitted drug mass, it measures nebulized volume without being able to distinguish solvent from active drug.

Aerodynamic properties of particles emitted by Sidestream were comparable to those found by Loffert *et al.*[19]. Even though FPF and MMAD allow a comparison of modes of nebulization for a tested product, they cannot be extrapolated to other medications or other conditions. These parameters are specific to each drug. Figure 27 shows a profile compatible with an optimal pulmonary deposition for SST used with 28.3 L.min^{-1}. At this same flow, the majority of particles emitted by the IPV are deposited on the two last stages, which is unfavourable for drug deposition into the lungs. As reported previously[127,159,160], when the entraining flow of the impactor increases, particles size decreases. As the filter for collection of particles with small size (lower than 0.7 µm) was extremely coloured during the nebulization by IPV, we postulate that a large quantity of the particles had a diameter lower than this size. This finding could eventually explain the difficulty to obtain measurable values of FPF and MMAD when using a high flow (60 L.min^{-1}). On extrapolation (Figure 28), the corresponding value for FPF with the IPV and a flow of 60 L.min^{-1} should be close to zero.

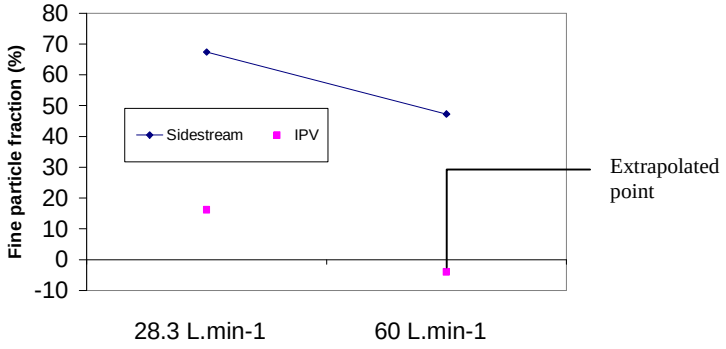

Figure 28: FPF obtained with both devices at an entraining flow of 28.3 L.min^{-1} and 60 L.min^{-1}

During spontaneous breathing, the inspiratory flow is close to 30 $L.min^{-1}$ and thus the results with 28.3 $L.min^{-1}$ are close to normal conditions of nebulization. Entraining flow of 60 $L.min^{-1}$ adapts better to experiments relating to metered-dose inhaler because patients are instructed to take a deep inspiration with this modality.

It should be noted that emitted dose of a nebulizer measured by impactor corresponds neither to the true emitted dose nor to the lung dose because it is necessary to take into account losses to the atmosphere and in dead space of devices as well as those related to extrathoracic deposition. One should rather speak about delivered dose.

Even though *in vitro* measurements cannot precisely predict pulmonary deposition, the deposition is directly related to size of the emitted particles[43]. Moreover, an European-French standard (EN-FN 13544-1)[1] of 2001 requires that all nebulizer manufacturers follow this kind of *in vitro* procedure.

Interestingly, the function of nebulization of a widespread technique such as the intrapulmonary percussive ventilation has not been evaluated previously. Homnick *et al.*[161] carried out a comparative study of IPV and conventional chest physiotherapy combined with a nebulization of albuterol. However, the function of nebulization was not evaluated specifically, which complicates interpretation of data. Confounding factors like associated treatments (percussions, positive pressure and conventional physiotherapy) do not make possible to distinguish the individual influences on the results obtained from this single study. Modification of mucus, which was one of selected means of evaluation, is not the principal effect expected following nebulization of albuterol. The study population size was small and included patients with a large range of age varying from 2 to 40 years. Pulmonary status was also greatly variable (FEV1 ranging between 34 and 95 % of predicted values). Accordingly, it has been clearly demonstrated that both age[162] and pulmonary status[134] influence pulmonary deposition. A larger and more homogeneous study population would be required for optimal evaluation of function of nebulization in healthy volunteers.

In conclusion, our results are unfavourable to IPV and predict a poor lung deposition. *In vivo* studies are necessary to confirm *in vitro* data. IPV should be subjected to the same requirements as any other nebulizer if it is used for drug nebulization.

C. Study of the aerodynamic properties of the IPV by the laser diffraction

1. Material and Method

Aerodynamic properties of Sidestream (MedicAid, Brussels, Belgium) and IPV1 (Percusionnaire, Brussels, Belgium) nebulizers were tested by laser diffraction by means of a Malvern Mastersizer X (Malvern Instruments, Inc., Southborough, MA). Experimental scheme was similar to that explained in the previous experiment.

A monodose 2.5 ml solution of a mixture of ipratropium (0.5 mg) and salbutamol (2.5 mg) was nebulized during one minute through laser beam. Either nebulizers were used in their traditional configuration with the original mouthpiece. The measured parameters were FPF and MMAD.

Three different Sidestream nebulizers were tested twice. IPV was used with four different frequencies of percussions and without superimposed percussion. On each frequency, four measurements were carried out. Either nebulizers were connected to an air cylinder. The pressure of the compressed air was of 4 bars.

To check for differences in MMAD and FPF with different models of SST, values were compared using repeated-measures analysis of variance (ANOVA). The same method was followed with different adjustments of IPV with superimposed percussions. Both parameters were analyzed using the independent Student t-test and were compared between SST and IPV with and without superimposed percussions. Statistical significance was assumed for $p<0.05$.

2. Results

No difference in particle size analysis was observed between different models of SST ($p = 0.27$ and $p = 0.15$ for MMAD and FPF respectively).

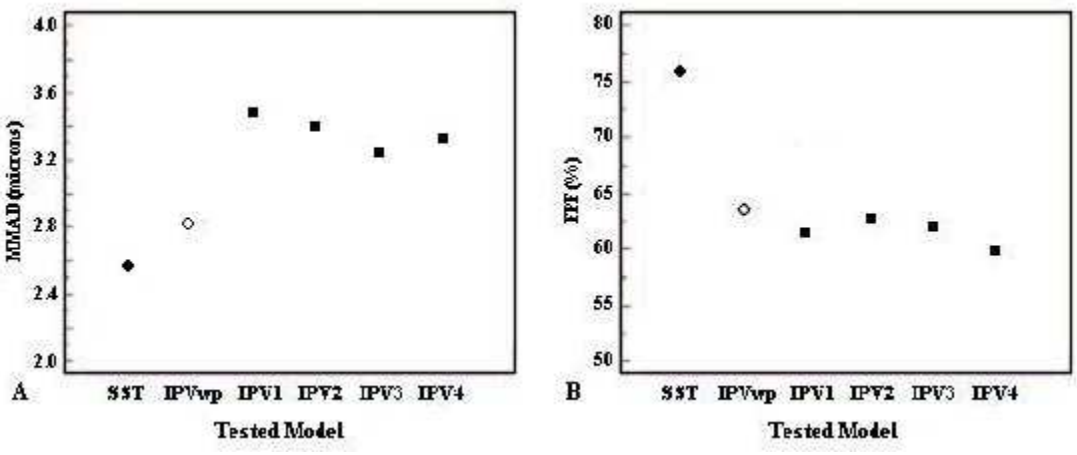

Figure 29: Aerodynamic particle size analysis obtained for Sidestream (SST) and Intrapulmonary percussive ventilation (IPV). (A) Mass median aerodynamic diameter (MMAD) expressed in microns. (B) Fine particle fraction (FPF) expressed in percentage. Values are mean of 3 different models for SST. IPV values were obtained under superimposed percussions (IPV1 to IPV 4) and without percussions (IPVwp)

Interestingly, an intense relationship is overall observed between mass median aerodynamic diameter and fine particle fraction (Figure 29). Apart from adjustments of the frequency of percussions, IPV generates highest values of MMAD and lowest value of FPF. Adjustments of IPV with superimposed percussions do not induce any modification in particle size parameters ($p = 0.34$ and $p = 0.16$ for MMAD and FPF, respectively). However, a significantly lower MMAD is obtained for IPV without percussion as compared to that monitored from different frequencies of superimposed percussions ($p < 0.001$) and for SST compared to that obtained for IPV without superimposed percussion ($p < 0.001$).

Without superimposed percussions, FPF is significantly higher comparatively to adjustments with percussions ($p = 0.02$). Comparisons between Sidestream and IPV show that FPF is significantly higher for Sidestream than for IPV with or without percussion ($p = 0.001$ and $p < 0.001$ respectively)

D. Global discussion on comparative *in vitro* studies

These results differ surprisingly between the two studies. Cascade impaction gives smaller MMAD and FPF for IPV comparatively to SST whereas by laser diffraction,

although FPF remains lower, MMAD is higher with IPV than with SST. Laser diffraction shows that the percussions have a greater effect on MMAD than on FPF. What could be the reasons of these differences?

A first reason that could be raised concerns the absence of mouthpiece in the case of the impactor. Accordingly, it was previously demonstrated that mouthpiece can influence the deposition[51,114]. A major difference between the two techniques relies on the concept of mass and volume. Although Andersen impactor may give precise information on the collected mass on its various stages and thus on particles size of drug solution, analysis by laser diffraction provides information related to the total volume of the solution (including solvent). Then laser diffraction does not determine the mass of drug which may affect the results, particularly with suspension formulations where no drug may be contained into droplets[45].

Another possible explanation could be the contribution of the entraining flow used with Andersen cascade impactor due to evaporation phenomenon. Dolovich[162] showed that the volume median droplet diameter measured by laser diffraction was 1–2 μm greater than the mass median aerodynamic diameter obtained by cascade impaction, and the difference was attributed to evaporation in the impactor. A slower entraining flow as found in laser diffraction was then proposed but even with this precaution, evaporation is likely to bias the results[163]. A study did not observe any difference in MMAD when comparing Andersen impactor to laser diffractor and when the collector was cooled to avoid this phenomenon[164]. It suggests that droplet distribution data for wet aerosol, where evaporation process has not been minimized, must be viewed with caution.

Finally, a third element can contribute to explain differences observed in our results. The type of inlet may also play a role in the results. Indeed, there are many different designs of inlet (Figure 30) and their choice can significantly influence the dose sampled with the cascade impactor[45].

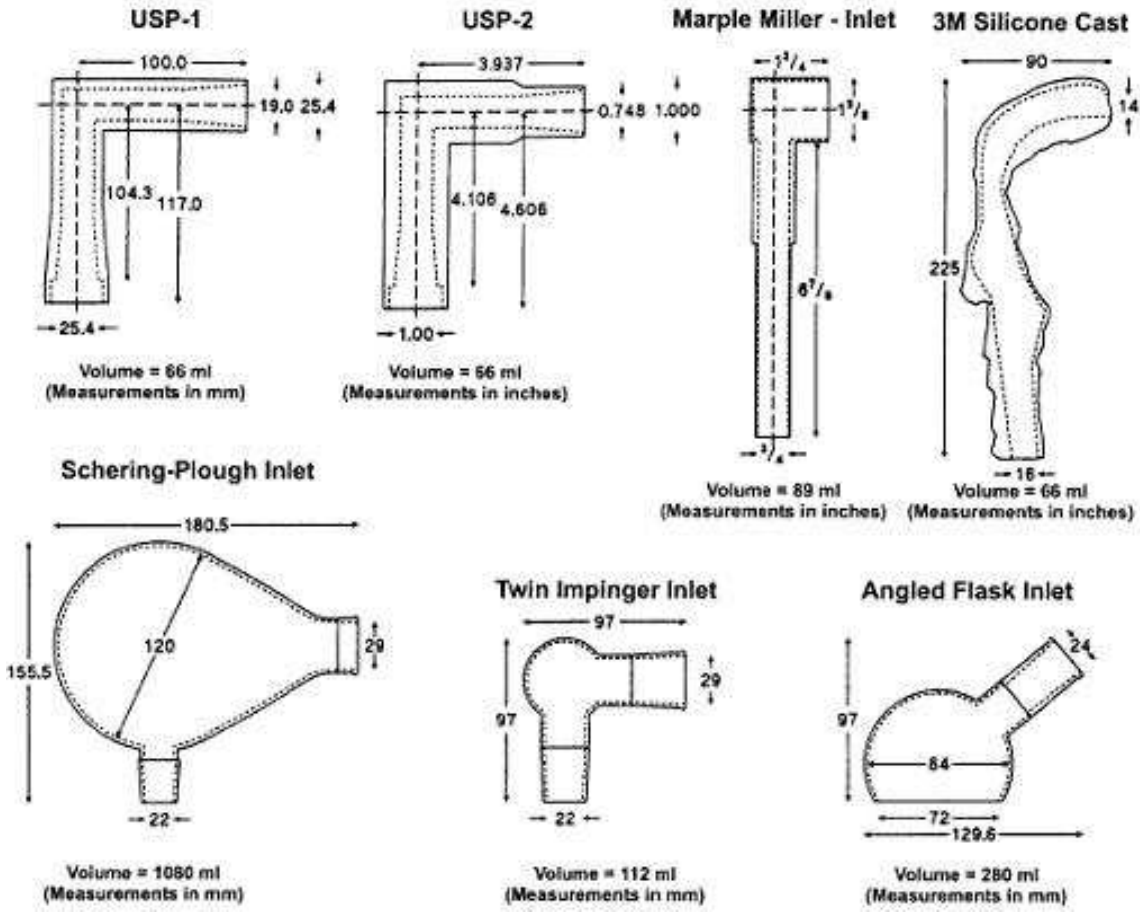

Figure 30 : Several inlets used with cascade impactors[45]