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Effect of Humidity on Constant Output and Breath Enhanced Nebulizer Designs When Tested in the EN 13544-1 EC Standard

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Aqueous aerosols produced by nebulizers used in clinical situations can rapidly change size as the patient inhales. This is caused by air with a relative humidity (RH) lower than inside the nebulizer being entrained and mixed with nebulized aerosol during the inhalation maneuver. A way to assess the change in size is to measure the aerosol in a test method that reflects the clinical situation. The EC standard, EN 13544-1, offers a first step towards this assessment. In this paper we have tested two nebulizer designs, one conventional constant output nebulizer and one breath-enhanced nebulizer, using the proposed standard in order to assess the effect of the relative humidity of entrained ambient air on nebulized aerosol size properties. The results indicate that aerosol size from the conventional nebulizer is greatly affected by the RH of the entrained air, while the breath-enhanced nebulizer is not affected. The results agree with theoretical expectations of how the entrained air interacts with nebulized aerosol. In the breath-enhanced nebulizer, the air is passed through the nebulizer interior and becomes saturated with moisture drawn from the relatively large nebulizer reservoir solution. With the conventional constant output nebulizer, ambient air is drawn over the nebulizer and draws its moisture from the relatively small volume of nebulized aerosol released from the nebulizer. For the conventional nebulizer design, we found a large decrease in measured nebulized aerosol size with decreasing relative humidity—3.1 μm MMAD at 75% RH fell to 1.9 μm MMAD at 20% RH. For the breath-enhanced nebulizer design, the MMAD was stable between a similar humidity range. The results indicate that aerosol size is dependent on relative humidity of the entrained air for the constant output jet nebulizer design that has no air entrainment through the nebulizer. We found no significant effect of ambient humidity of entrained air on nebulized aerosol size from the breath-enhanced nebulizer design.

INTRODUCTION

Aerosols are often used in inhalation therapy in the medical field to administer drugs in aerosol form locally to the lungs. A common way to administer the aerosols is by nebulization of aqueous drug formulation using a jet nebulizer. The jet nebulizer produces large numbers of water droplets of the aqueous formulation. It’s droplet size and output behavior has been investigated earlier by authors such as Mercer (1965) and Newman (1986). It is also apparent that the droplets are volatile and that they can rapidly change their size depending on the ambient conditions in which they are released (Ferron and Soderholm 1990; Ferron et al. 1997). Recently more thorough investigations have been performed regarding nebulizer characteristics whereby the behavior influenced by water evaporation and air humidification has been discussed (Smye 1991; Prokop et al. 1995; Stapleton and Finlay 1995; Phipps and Gonda 1990). These authors have shown using different approaches that the gravimetrically determined output is the sum of nebulizer solution and water vapor in the flow of air that is used to operate the nebulizer. The vapor arises from evaporation occurring at the liquid surfaces inside the nebulizer. The driving force is the humidification of the air used to operate the nebulizer in the direction of saturation, which in turn cools the nebulizer and the bulk liquid (Phipps and Gonda 1990). Stapleton and Finlay (1995) has performed a more thorough investigation of the evaporation process along with mathematical modeling where the evaporation both from the bulk liquid and droplet surface has been accounted for. Smye (1991) has mathematically predicted the “respirable” fraction of drug-containing aerosol for a typical nebulizer from its output characteristics. All in all there is a good understanding of the output characteristics of nebulizers and the solvent/solute output and evaporation process involved. However, nebulizers are seldom
used as stand-alone devices. They are often used in clinical set-ups that allow for patient breathing cycles (e.g., inhalation and exhalation), thereby allowing for air entrainment. In a typical clinical set-up, changes in the ambient conditions can be expected as air with a relative humidity (RH) lower than inside the nebulizer is entrained in the set-up during inhalation. The RH of the entrained air much depends on the geographical location and time of the year when the set-up is used.

Characterization of the nebulizer aerosol is often confined to measuring the aerosol at the nebulizer exit by laser diffraction methods (Knoch 1994; Clark 1995; Phipps and Gonda 1990). This methodology does not take into account the changes occurring during the in vivo situation where extra tubing and mouthpieces are used together with air entrainment. A preferred methodology would be to size the aerosols by means of their aerodynamic characteristics, as this describes the behavior of the particles in the aerosol as they are carried and deposited in the airways of the lung. One of the common techniques that can be employed is the cascade impactor, which uses particle inertia (e.g., aerodynamic) properties when collecting and sizing the particles of an aerosol distribution.

So far the aerosol collection with impactors has been confined to solid particles or nonvolatile liquid droplets produced by a nebulizer in a steady airflow (Ho et al. 1986). Aerosol droplets from drug solutions are more volatile than oil droplets and therefore have not been considered for sampling in a cascade impactor. Many impactors used also sample all the air and thus aerosol that exits from the jet nebulizer, and further, they often need additional air to make up their operating airflow rate. When this additionally entrained air mixes with volatile nebulized aerosol, the amount of evaporation leading to reduction in particle size can significantly distort and dominate the measured particle size distribution. It has been proposed to sample the entire aerosol into an impactor after complete dryness (Roth and Gebhardt 1996), but that methodology does not mimic the in vivo situation. Impactors are also often difficult to connect in a clinically representative way without introducing unrealistically high airflows, which distort the true aerosol size distribution.

To avoid this problem, a sample can be drawn from the airflow of the set-up if a low flow cascade impactor with an operating air flow rate lower than the nebulizer in question is used. This has the advantage of accommodating sampling during both steady state and inhalation procedures. A low flow cascade impactor is also preferable as it has the advantage that it can be used in situ during the inhalation procedure. An in vitro method incorporating a low flow cascade impactor operating within a sinus flow breathing pattern has been applied (Smaldone et al. 1998). It has the advantage that it emulates a simple and well defined “in vivo” breathing situation. However, it has the disadvantage that the aerosol collected will be biased towards large unevaporated droplets generated in the first and last third of each sinus flow cycle (Dennis 2000).

The variable volumes of air with a certain RH entrained during the sinus flow can affect the nebulizer aerosol characteristics. An in vitro experiment with constant entrained air flow with a controlled RH is more appropriate in investigating the nebulizer aerosol characteristics. The design of jet nebulizer used and how it interfaces with the patient’s breathing pattern may also significantly influence the aerosol characteristics.

The European Community (EC) standard for type testing of nebulizers, EN 13544-1, describes reasonably clinically representative in vitro methodology to assess nebulized aerosol size using constant entrained airflow. The standard utilizes a low flow cascade impactor operated with an airflow rate of 2 L/min and a constant air-flow rate of 15 L/min. The Marple Personal Impactor series 290 (Westech Instrument Services Ltd., Bedfordshire, UK) has suitable characteristics. The aim of this paper is to investigate the effect of ambient humidity on nebulized aerosol size in different nebulizer designs when used in the set-up suggested in the EC standard. In this paper data on aerosol size distribution from a Marple series 296 personal impactor was used in the set-up specified in the European Standard on two jet nebulizers designs: a constant output design represented by the Microneb jet nebulizer (Lifecare Ltd., Leicestershire, UK), and a breath-enhanced nebulizer design embodied in the Pari LC+ jet nebulizer (Pari GmbH, Starnberg, Germany). The way in which these two nebulizer designs interact with entrained “inhaled” air is fundamentally different. They were expected to demonstrate fundamentally different droplet size dependence when entraining ambient RH in the set-up. The effect of RH was investigated by introducing “ambient air” in which the humidity had been controlled.

**MATERIALS AND METHODS**

**Experimental Design**

The experimental design used in this study was that specified in the EC standard EN 13544-1 for sampling and sizing of aerosols produced by commercial nebulizers. The aim of the experiment was to evaluate the effect of RH on nebulized aerosol size for two different nebulizer designs. The experimental set-up consisted of a nebulizer, a low flow cascade impactor fitted with t-pieces (Intersurgical, England), electrostatic filters (3M Filtrete type G, 3M, USA) with corresponding Pari (Pari GmbH, Starnberg, Germany) filter holders, and Bird (Bird, England) connectors. Commercially available tubing and t-pieces (Bird and Intersurgical, UK) were used as connectors in the rest of the set-up. Two nebulizers were tested in the set-up; the Microneb (Lifecare Ltd., Leicestershire, UK), a constant output nebulizer design; and the Pari LC+ (Pari GmbH, Starnberg, Germany), a breath-enhanced nebulizer design.

**Detailed Description of the Low Flow Impactor Set-Up Used for Both Nebulizers**

The effect of RH was investigated in both nebulizers by entraining ambient air with a controlled relative humidity of 20%, 50%, and 75% (Microneb), and 20%, 50%, and 90% (Pari LC+). Schematics of the experimental designs of the constant output
and breath-enhanced nebulizers are shown in Figures 1 and 2, respectively.

The Microneb and Pari nebulizers were filled with 2.5 ml and 5.0 ml of 2.5% NaF, respectively, and they were operated at airflow rates of 7.5 L/min (0.9 Bar) and 4.0 L/min (1.1 Bar), respectively. Dry pressurized air (≈1% RH, 22–23°C) was used to drive the nebulizers. Entrained air at controlled humidity of 20%, 50%, 75%, and 90% was supplied as shown in Figure 3.

The droplet size distribution exiting the nebulizer was measured for both nebulizers and used as an estimate of the initial size distribution unaffected by the RH. This was done using a Malvern Mastersizer and liquid in air presentation model. The nebulizer was fitted 0.5 cm from the laser beam and 3 cm from the lens. A 100 mm lens was used. The calculated Mass Median Aerodynamic Diameter (MMAD) of the results was found to be 5.6 μm for the Pari LC+ and 3.4 μm for the Microneb. These MMAD values were used as reference unaffected by the RH in the calculation performed.

General Methods

Impactor Assembly and Operation. Particle sizing was performed using a Marple Personal Impactor series 296 (WestTech Instrument Ltd., UK). The impactor was connected, as described in the EC standard, to a t-piece that allowed for sampling of the aerosol in an isokinetic manner. The impactor was assembled as described in the manual. The stages (6) were fitted with filter substrates cut from MGA type filters (Munktell, Sweden) together with a final filter (GF/A type, Whatman, UK). Aerosol found impacted on top of the first stage in this impactor was collected by an additional substrate labeled S0 placed on top of the first stage. It was operated at 2 L/min (adjusted by a bubble flow meter) using a central vacuum source and needle valve.

NaF Standard and Analysis. A stock standard solution of 2.5% w/v of NaF in distilled water was prepared. Analysis of the fluoride ion content in the nebulizer cup, tubing, MGA filter, and Pari filter was undertaken following washout. This was performed using a Marple Personal Impactor system.
done using an ion selective electrode (Model 96-09 combination fluoride electrode, Thermo Electron Corp., Waltham, MA, USA) connected to a meter Orion Model 720A ion selective meter (Orion Research Inc., USA) according to the methodology described by Dennis et al. (1990). A TISAB (Total Ionic Strength Adjustment Buffer) solution was made as a 10% solution in distilled water from stock TISAB III (Thermo Electron Corp., Waltham, MA, USA). The TISAB solution was added to fluoride standards using calibrated pipettes. The stock standard was used for a series of dilutions giving four standards of 0.3, 1.0, 10.0, and 100 μl (volume of initial 2.5% nebulizer solution per 10 ml TISAB solution).

The different parts of the set-up excluding the impactor were disassembled, washed with TISAB solution, and dried prior to each experiment. The nebulizers were filled with 2.5 ml or 5.0 ml of 2.5% NaF stock solution and nebulization commenced. The impactor was disassembled after nebulization and the impactor MGA filter substrates were transferred to a 50 ml plastic test tube type Falcon (Discovery Labware, Boston, MA, USA) and 10 ml of Tisab solution was added. The tubes were shaken well and left for 15 min to allow for extraction and dissolving of the NaF particles deposited.

All results were calculated as μl of 2.5% NaF solution nebulized per minute or in total.

Humidity Control. The relative humidity of the entrained air in open systems was measured with a General Eastern model Hygro-M2 control unit and a 0111D RH cell (General Eastern, Plainville, CT, USA). A system for generating a flow of air with a relative humidity (RH) ranging from 20% to >90% was constructed. The system consisted of a fully humidified (≈100% RH) airline and a dry line (e.g., compressed air, RH ≈ 1%). The air from the each line was mixed and 1 L/min of the air mixture was passed through the General Eastern humidity sensor. When adding air at RH above 80%, the bottle had to be temperature adjusted in a water bath until a stable RH was reached. The system used to generate and monitor relative humidity of “entrained air” is shown in Figure 3.

Flow Rate Monitoring. All airflow rates were monitored using either a Mini-Buck M-5 calibrator (A.P. Buck Inc., Orlando, FL, USA) or a rotameter 1-25 L/min (Solatron Mobrey Ltd., Slough, Berkshire, UK) calibrated by the bubble flow meter. The flows were corrected for temperature and pressure deviation from the standard atmosphere and they were measured before and after each cascade impactor run.

Temperature Monitoring. The temperature was measured using two calibrated Pronto type PTS10501K (Thermo Electric Co. Inc., Saddle Brooks, NJ, USA) temperature measurement units equipped with small thermocouple temperature elements. The elements were fitted in the aerosol flow at the exit of the nebulizer and at the inlet of the impactor and shielded from direct impaction of liquid aerosol by using a small plastic cover. The temperature was measured from start of nebulization to end of nebulization, e.g., 120 s for the Pari LC+ and 180 s for the Microneb. Measurements were taken every 30 s and they were done in the same way for both nebulizer set-ups. One measurement per RH and nebulizer was performed. The ambient
temperature and the temperature of the entrained were measured and were found to be 23°C ± 1°C.

Theoretical Calculation of the Expected Droplet Size Distribution. A theoretical estimation on the expected droplet size distribution sampled at the impactor inlet at different RH was done using the equations proposed by Stapleton and Finlay (1995). The temperature of the entrained air was 23°C and the RH of the nebulizer driving air was assumed to be 1%. The temperature of the air exiting the nebulizer and the air entering the impactor was derived from the temperature monitoring and the mean of the temperature drop was used as an estimate in the calculations. The calculations of the water holding capacity and the content of water in the surrounding air prior to evaporation gave an estimation of the water that could be transported as vapor to the surrounding air and the droplet size decrease obtained. The calculations were applied using the MMAD as an estimate and were performed for 20%, 50%, and 75% RH for the Microneb and at 20%, 50%, and 90% RH for the Pari LC+. It should also be noted that the calculations assume that equilibrium has been reached and no significant changes in droplet sizes are occurring.

RESULTS

The purpose of this study was to examine the effect of RH of the entrained air on the droplet size distribution sampled by the Andersen 296 impactor for a constant output design nebulizer and a breath-enhanced design nebulizer when used in the EC standard EN13544-1.

The temperature drop at the nebulizer output and at the impactor inlet was measured for both nebulizer set-ups and for entrained air of 20%, 50%, and 75% RH. The entrained air had a temperature of 22–23°C during all experiments. This was done during the first 2 min for the Pari LC+ and during the first 3 min for the Microneb and in 30 s intervals for both nebulizers. The nebulizer driving air had a RH of 1% and a temperature of 22–23°C. The results can be seen in Figures 4 and 5 for the Microneb and Pari LC+, respectively. The Pari LC+ had a smaller temperature decrease than the Microneb at the same temperature, both at the nebulizer exit and at the impactor inlet. The Microneb had the same temperature at the nebulizer exit for all three RH settings. In general, higher RH gave a smaller temperature drop over time as compared to low RH.

Constant Output Nebulizer

The gravimetrical output and the NaF solute output (by filter fitted on the nebulizer outlet) of the Microneb nebulizer was initially measured. The loss of solvent (water evaporation from the reservoir) when operating the nebulizer with dry air was estimated to 160 µl/min during the first 60 s as the gravimetric output was 300 µl/min and the measured NaF solute output was 140 µl/min.

The Microneb nebulizer was operated with 7.5 L/min airflow through the nebulizer, which was entered through the t-piece and introduced into the system. The sampling time was 120 s. Three different RHs of the entrained air were used: 20%, 50%, and 75%. The MMAD was found to be 3.1 µm at an RH of 75%, 2.7 µm at an RH of 50%, and 1.9 µm at RH of 20% (see Figure 6 and Table 1). The decrease in MMAD is not linear with decreasing RH.

![Microneb temperature decrease](image_url)

Figure 4. Microneb temperature profile during 180 s nebulization.
Breath-Enhanced Nebulizer

The NaF solute output of the Pari nebulizer was measured in the same way as for the Microneb nebulizer. The amount of NaF found on the Pari excess air filter increased from 140 μl/min to 410 μl/min when entraining air through the Pari nebulizer top as compared with the top valve closed. The former value was obtained during the Malvern measurement.

The nebulizer was operated with an airflow rate of 4 L/min through the nebulizer. 11 L/min entrained air was added in all experiments. Entrained air of 90%, 50%, and 20% RH was let through the nebulizer. The nebulizer was set up as shown in Figure 6.

Figure 5. Pari LC+ jet temperature profile during 120 s nebulization.

Figure 6. Microneb set-up with 11 l/min entrained air.
Table 1
Summary of results for the Microneb and Pari LC+

<table>
<thead>
<tr>
<th>Design</th>
<th>System</th>
<th>RH (%)</th>
<th>MMAD (μm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conventional</td>
<td>Micro_open</td>
<td>75</td>
<td>3.1</td>
</tr>
<tr>
<td>design (Microneb)</td>
<td></td>
<td>50</td>
<td>2.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20</td>
<td>1.9</td>
</tr>
<tr>
<td>Breath-enhanced</td>
<td>Pari_open</td>
<td>90</td>
<td>5.6</td>
</tr>
<tr>
<td>design (Pari LC+)</td>
<td></td>
<td>50</td>
<td>5.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20</td>
<td>5.5</td>
</tr>
</tbody>
</table>

through the nebulizer top valve in the experiments. The sampling time was 120 s. There was virtually no difference in the droplet size distribution for all experiments performed at different RH of the entrained air (Figure 7 and Table 1). The MMAD of the Pari was found to be 5.6 μm at 90% RH, 5.6 μm at 50% RH, and 5.5 μm at 20% RH.

**DISCUSSION**

According to Ferron et al. (1997) the droplet size changes rapidly while still inside the nebulizer. The RH after production is assumed to be about 99.5%, causing droplets below 2 μm to reach their terminal size within 0.1 s. This is caused by evaporation of the aerosol particles and the liquid contained inside the nebulizer. The terminal size for a 2 μm aerosol will be reached inside the nebulizer assuming that a typical nebulizer internal volume is about 40 ml and the typical flow rate being 6000 ml/min. The result is a high total output (aerosol + water vapor), as compared to drug aerosol output alone, and is well known (Dennis et al. 1990). The time to reach the terminal size (droplet or particle), according to Ferron et al. (1990), depends on the RH and the initial droplet size. A 1 μm NaCl droplet will reach its terminal size within 0.015 s at 95% RH, and this time will decrease with decreasing RH. The time for a 10 μm salt droplet to reach terminal size is about 2 s at 99.5% RH and falls to 0.15 seconds at 50% RH. Solid salt particles start to form at 40% or less (Ferron et al. 1990). The effect of high concentrations of droplets in a distribution may extend or decrease the evaporation time due to local high RH and temperature drop/increase around the droplets located in the aerosol cloud, which in turn results in one- or two-way coupling of the mass transfer of vapor (Finlay 1998). The treatment of the aerosol evaporation behavior as one- or two-way coupled depends on the droplet/vapor ratio and the surface area exposed by the droplets in the air (Finlay 1998). Turbulent mixing (as expected in the EC standard) can also lower the evaporation times further (Ferron et al. 1990). A set-up to measure the equilibrium state of aerosols leaving a typical nebulizer has been described by Stapleton and Finlay (1995), where it is shown that equilibrium is reached after exit from the described nebulizer and is maintained in the system described. The length of the set-up described and the residence time of the aerosol in the set-up can be estimated to be in the

![Figure 7. Pari LC+ set-up with 7.5 l/min entrained air.](image-url)
second interval. As the residence time in the system was calculated to be 0.5 s, it can be concluded that an equilibrium droplet size distribution of the investigated nebulizers can theoretically be reached when sampled by the impactor.

The evaporation effect was estimated for the Microneb nebulizer in this study. This nebulizer had a gravimetric output of 300 μl/min and a measured NaF solute output of about 140 μl/min. The loss of solvent (water evaporation from the reservoir when operating the nebulizer with dry air with a RH of 5%) could then be estimated to 160 μl/min during the first minute. The evaporation causes the temperature to drop inside the nebulizer, and the aerosol-laden air leaving the nebulizer cools down. This can be seen in Figure 4 for the Microneb nebulizer.

However, less well appreciated is the effect of evaporation of nebulized aerosol droplets postproduction and the aerosol release from constant output nebulizers. When the aerosol exits the nebulizer in Figure 1 it meets the ambient entrained air that has entered the t-piece fitted on top of the nebulizer. This is a situation that is common for most constant output nebulizers such as the Microneb.

Consider the following typical case for the above nebulizer type: The entrained air, having a temperature of about 20°C and a RH of 50%, mixes in the t-piece with a relatively small amount of cooler aerosol containing air with high RH that is released from the top of the nebulizer. When mixing the nebulizer air temperature increases while the ambient air is cooled down, which results in a new temperature level. Evaporation can be expected as the humidity of the entrained air is far from saturated and as the aerosol-laden air with a high RH increases its water holding capacity as the temperature increases. Supposing that the water holding capacity of the entrained air is relatively high compared to the total volume of liquid aerosol surface available, the droplets rapidly shrink in size—this is especially pronounced when the humidity of ambient entrained air is low (e.g., high water holding capacity). The extent of evaporation depends on the RH of the ambient air and the temperature difference between the aerosol-laden air and the entrained air.

Fully humidified air at room temperature (e.g., 20°C) requires some 17.3 μl of water vapor per liter of air. If the total entrained air flow is around 10 L/min, this air will then theoretically be capable of absorbing 173 μl of water vapor per minute in total if it had a low (zero) initial humidity. Even if the humidity was 50% RH, some 90 μl can be absorbed. These numbers are significant compared to the total nebulized aerosol output from commonly available nebulizers that vary typically between 100–300 μl (Roth and Gebhardt 1996).

In contrast, breath-enhanced nebulizer designs do not mix ambient air with released aerosol. Instead, entrained air enters the main nebulizer reservoir chamber. When using the Pari LC+ nebulizer the entrained air is added through the top flap valve arrangement of the nebulizer (see Figure 2). As a consequence, no external t-piece delivering entrained air was required in the experimental design. It then passes the generation point, where it is humidified by the generated droplet aerosol and simultaneously mixed. The humidification causes the temperature to drop inside the nebulizer in the same way as for the Microneb constant output nebulizer. This temperature drop for the Pari LC+ can be seen in Figure 5. Even if the entrained air has a low relative humidity the vast surface area and massive production of droplets in the nebulizer reservoir ensures a high water vapor potential by evaporation. Most of the aerosol initially produced by the nebulizer jet is also recycled in the nebulizer, and only about 1% (the smallest droplets) escapes the baffle structures of the nebulizer (Nerbrink et al. 1994). The volume of the nebulizer reservoir is relatively large (typically >1000 μl) compared to the volume of the absorbed water vapor required to humidify the 10 L/min or so of entrained air. All of the air exiting the nebulizer will therefore have a high relative humidity close to saturation and a fairly stable droplet distribution.

**Effect of RH on the Droplet Size Distribution: Constant Output vs. Breath Enhanced**

Though both the Microneb and Pari nebulizer designs are driven by compressed air entering the nebulizer from its base, the manner in which they entrain and supply make-up air for patient inhalation is fundamentally different. This difference has important implications when measuring the released aerosol size droplet distribution.

**Constant Output Nebulizer.** The MMAD of the droplet size distribution exiting the Microneb nebulizer at various relative humidity of entrained air was as follows: 3.1 μm for an RH of 75%, 2.7 μm at an RH of 50%, and 1.9 μm at RH of 20% (Figure 6 and Table 1). The MMAD does not decrease linearly with decreasing RH, which is expected, as the droplet diameter is a cube root function of the droplet volume. It should also be noted that the MMAD is not a very good measure of the evaporation in this case as the effect seen on the droplet is strongly size dependent and smaller droplets proportionally change size more per unit time than larger droplets (Hinds 1982). This would increase the GSD of the distribution, and effect can be seen already at an RH of 75%. According to Hinds (1982) and Ferron and Soderholm (1990), the stabilization times of droplets of 1 μm or less is in the ms range for a wide range of RH, which is lower than the residence time of the droplets in the system (estimated to be 0.5 s). These droplets should therefore have evaporated to a stable size before being collected by the impactor. The RH dependence of the stabilization times for droplets larger than 1 μm in a droplet size distribution may already be evident at a RH of 75%, as it can be expected that predominantly the smaller droplets have evaporated significantly and possibly stabilized, thereby slightly shifting the distribution towards smaller sizes (Figure 6).

Ferron et al. (1997) has shown for three nebulizer brands that 95% of the droplets in a distribution have reached a stable size when leaving the nebulizer. The air surrounding the droplets has a RH of about 99.5% when leaving the nebulizer. When this emitted droplet aerosol meets entrained air with a RH lower
The effect on the droplet size distribution caused by entraining air through the Pari breath-enhanced nebulizer design was stable, which was found to be in

<table>
<thead>
<tr>
<th>RH (%)</th>
<th>Microneb</th>
<th>Pari</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MMAD experimental</td>
<td>MMAD calculated at 23°C</td>
</tr>
<tr>
<td>99.5</td>
<td>3.4</td>
<td>5.6*</td>
</tr>
<tr>
<td>90</td>
<td>N/A</td>
<td>5.5</td>
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<tr>
<td>50</td>
<td>2.7</td>
<td>N/A</td>
</tr>
<tr>
<td>20</td>
<td>1.9</td>
<td>5.5</td>
</tr>
</tbody>
</table>

*Values for 99.5% RH derived from Malvern Mastersizer measurements.

than 99.5% in a t-piece, they start to evaporate. This evaporation process, when using conventional nebulizers, can be estimated to have two-way coupling behavior if the RH is lower than 90% according to Finlay (1998). The two-way coupling would slow down the evaporation process and equilibrium would be reached later than if the behavior could be referred to as one-way coupled. The timescale considered by Finlay (1998) is 0.1 s, which is 5 times shorter than the residence time of the droplet aerosol in the EC standard set-up. It is therefore possible that the evaporative processes experienced by the droplets in the aerosol are so small when they reach the impactor inlet so that they would not affect the sampling by the impactor to a large extent. The smaller droplets would adapt to RH changes fast (ms), while the larger droplets would take longer time to adapt (Hinds 1982), which could skew the distribution sampled by the impactor. The observed increase in temperature from the nebulizer outlet to the impactor inlet implies that the water holding capacity of the air increases as the aerosol traverses through the set-up and that evaporation process is ongoing.

Air can hold 17.3 μl water vapor per liter at an average air temperature of 20°C. With a total output of 300 μl/min from the Microneb and 160 μl/min of water vapor leaving the nebulizer it is clear that the air inside the set-up can not accommodate this amount of liquid at any of the RH investigated or the temperatures observed. The aerosol cannot completely dry out and will therefore contain some water.

It is also well known (Phipps and Gonda 1990) that the temperature decreases with time when nebulizing, which was observed for the Microneb nebulizer and can be seen in Figure 4. It can also be noted that the temperature increases as the aerosol is transported to the impactor inlet. The temperature drop when nebulizing is independent of the RH of the entrained air, which can be expected as the air entrainment occurs at the t-piece that is located after the nebulizer exit. The temperature increase at the impactor inlet depends on the initial temperature and RH of the entrained air, being highest for the high RH and lowest for the low RH if assuming the same temperature. This can be explained by the temperature drop caused by evaporation of the aerosol droplets to the surrounding air. The surrounding air has a higher relative water holding capacity to reach 99.5% RH when entraining low RH air as compared to high RH air. A theoretical estimation of the expected droplet size distribution sampled at the impactor inlet at different RH was done using the equations proposed by Stapleton and Finlay (1995). The results can be seen in Table 2. A good agreement between the experimental data and calculated data is seen for all RH except 20%. An explanation to this may be that the evaporation process starts to change the size of the the larger droplets more noticeably and that the smaller ones are stabilized at this RH. This would narrow the distribution and give a smaller mean diameter of the size distribution (Hinds 1982).

Another explanation could be the latent heat of the impactor. If the impactor is kept at ambient temperature (e.g., warmer than the aerosol entering the impactor) it would heat the aerosol, thereby continuing the evaporation process further. This phenomenon has been described by Stapleton and Finlay (1998) and would be predominant for the 20% entrained air as the cooling of the aerosol-containing air by evaporation between the t-piece and impactor inlet is substantial in this case (Figure 4). Applying the equations proposed by Stapleton and Finlay (1995) and using an ambient temperature (23°C) in the impactor shows an effect of decreasing MMAD as compared to the calculations performed using the mean temperatures measured at the impactor inlet. The effect was most pronounced for low RH of the entrained air (Table 2). There was an effect at higher RH as well but less pronounced as the temperature difference between ambient (e.g., entrained air) and impactor inlet is smaller than for low RH. One should also bear in mind that Stapleton and Finlay (1998) collected data when the nebulizer had stabilized at a lower temperature (e.g., long running time) and that the larger Andersen MKII impactor was used, a device having much larger total latent heat than the impactor used in this study.

Breath-Enhanced Nebulizer. The effect on the droplet size distribution caused by entraining air through the Pari breath-enhanced nebulizer design was stable, which was found to be in
direct contrast to the Microneb constant output nebulizer design. As can be seen from Figure 7, the distributions are nearly constant when changing the RH of the entrained air. The entrained air is humidified when passing through the internal parts of the nebulizer and thus has little, if any, effect on the particle size of released aerosol, as all aerosol released is shrouded in a fully humidified air stream and is therefore size stable. The air is also inside the nebulizer for a substantial time period compared to the ongoing evaporative/humidifying processes. Stapleton and Finlay (1995) came to a similar conclusion for the Pari LC+ nebulizer when entraining air (28.3 l/min total flow, nebulizer and entrained air) through the nebulizer. They concluded that more than 99% of the mass is in equilibrium at the exit point of the nebulizer.

The temperature at the exit of the nebulizer and at the impactor was monitored, for the three different settings of 20%, 50%, and 75% RH of the entrained air, see Figure 5. It can be noted that the temperature increases as the aerosol was transported to the impactor inlet just as was seen for the Microneb nebulizer. In contrast to the Microneb, the temperature drop when nebulizing was found to be dependent of the RH of the entrained air, which could be expected as air is entrained through the nebulizer internal parts. The degree of cooling of the air containing aerosol inside the nebulizer depends on the extent of evaporation needed to humidify the air to equilibrium (RH ~99.5%), which in turn depends on the RH of the entrained air. The lower the RH, the more evaporation, which in turn leads to a lower temperature. This is evident in Figure 5.

An estimation of the water vapor release from the aerosol droplets was performed for the Pari nebulizer in the same way as for the Microneb nebulizer. Calculations on the expected droplet size distribution sampled by the impactor were performed using the equations of Stapleton and Finlay (1995). The calculations were done for 20%, 50%, and 90% RH, applying the calculations on the MMAD. The mean temperature used for the air entering the impactor was derived from Figure 5. Calculations of the water holding capacity and the content of water in the surrounding air prior to evaporation gave an estimation of the water that could be transported as vapor to the surrounding air and the droplet size decrease obtained. The results can be seen in Table 2. The calculated results are in good agreement with the experimental data and indicate a small shift in the diameter of less than 3% from the estimated initial size produced by the nebulizer (calculated from Malvern measurements). This is also in good agreement with the results obtained by Finlay (1998), who concluded that the Pari LC+ can be approximated as a stable aerosol when entraining air with RH ranging from 15% to 90% through the top valve. He also concluded that the mass of water in droplets was significantly larger than the mass of water in the vapour phase, so that the evaporative effects changing the droplet size distribution would be negligible and there would be no need for one- or two-way coupled mass transfer calculations.

The droplet size distribution would probably be unaffected when sampled in the impactor as the evaporation of water from the droplets was estimated to be less than 3% at the point where they enter the impactor. Evaporation would affect the smaller droplet diameters most, thereby widening the distribution at the lower size range, and these droplets would be collected on impactor plates with a smaller cut of diameter than they should have been collected on. All droplets would on average contain water and the impactor plate substrates should therefore contain liquid from these droplets.

An estimation of the effect of impactor latent heat was also performed the same as was that for the Microneb nebulizer. It was performed as a worst case calculation by setting the temperature to 23°C in the impactor and performing the calculations again on the three humidity settings. The results are shown in Table 2 and show that there would be an 11% decrease in the droplet diameter for a RH of 20% and 4% for a RH of 90%. This would clearly be visible in the results obtained from the impactor sampling and is not supported by the data observed (Figure 6 and Table 1). Again the effect may not be as pronounced as suggested by Stapleton and Finlay (1995), as the aerosol sampled by the impactor was released during the first 2 min of nebulizer operation and starting at ambient temperature conditions. Stapleton and Finlay (1995) waited until the nebulized aerosol had stabilized in temperature drop, which occurred after 4 min of operation. Other authors like Kwong et al. (2000) have shown that there is a latent heat effect on the Andersen 290 series impactor, but the set-up and operating conditions are substantially different compared to this study.

The amount of NaF found on the Pari excess air filter increased from 140 µl/min to 410 µl/min when entraining air through the Pari nebulizer top as compared with the top valve closed. The increase in output, and slight shift to smaller droplet sizes when entraining air as compared to the initially produced droplet size distribution, is most likely explained by the air flow changes induced by the entrained air. The primary produced aerosol is the same in both cases (closed valve/entrained air). The airflow past the internal parts of the nebulizer increases when air is entrained and the total air flow through the system increases. This increased air flow can carry more droplets out of the nebulizer per unit time considering that the droplet density is limited to 10⁶/cm³ by coagulation. The entrained air flow may even act as a virtual impactor, being perpendicular to the baffle system inside the nebulizer and thus drawing the produced aerosol away from the baffle system and internal surfaces. An increased aerosol output may be expected as a result of both phenomena described above. The implications of the above increase in solute output when entraining air through the nebulizer is important from a clinical view. The patient inhalation maneuver varies during an inhalation treatment. This will vary the amount of entrained air and thereby vary the dose given per breath. This variation will have an overall negative effect on the consistency of the total dose given.
Sampling and Deposition Considerations in the Systems

There is also evidence that the aerosol droplet distribution is sampled correctly for both nebulizers tested according to the setup described above as the NaF mass ratio impactor/excess filter corresponds to the respective air flow rates. The two nebulizers used in this investigation, having quite different droplet size distributions, showed an expected percentage of the mass ratio (13.4% of the total mass collected at 2 L/min) when a filter substituted the impactor at the t-piece connection. This implies that sampling is correct and that the sampling can be seen as isokinetic for all droplet size distribution generated by these two nebulizers. If sampling problems were evident a value shift from the optimal 13.3% would be expected.

When calculating the Reynolds number of the flowing air in the t-piece and tubing it is found that the flow is predominantly turbulent. An indication of deposition can be given by looking at the stopping distance for the larger droplets generated by nebulizers. The stopping distance and the sampling Stokes number can be calculated for flows in the t-piece assuming an aerodynamic droplet size of 10 \( \mu m \), \( V_{\text{mean}} \) of 66 cm/s, and a Reynolds number of the particle of 0.6 according to Hinds (1982). The stopping distance will be 0.2 mm and the Stokes number 0.01. The stopping distance is small compared with the internal dimensions of the tubing (~20 mm), and the Stokes sampling number is close to where sampling problems may arise.

CONCLUSIONS

In this paper, the methodology adapted from the European nebulizer standard (EN 13544-1) incorporating a system for controlling humidity was used to investigate the effect of humidity on nebulized droplet size from two nebulizer designs (constant output and breath enhanced).

The results indicate that the characteristics of the aerosol sampled to the impactor depends on the relative humidity, RH. These variables can change drastically depending on the nebulizer tested and how the set-up is configured and will thereby change the aerosol size distribution accordingly. For the constant output nebulizer design tested, the Microneb, the change in droplet size distribution was found to be 3.1 \( \mu m \) MMAD at 75% RH, which fell to 1.9 \( \mu m \) at 20% RH. In contrast, the Pari LC+ breath-enhanced nebulizer design showed no effect from RH. Calculations based on equations proposed by Stapleton and Finlay (1995) have been used to explain the observed behavior with varying RH. The implications of the above are important when considering the implications of the effect of ambient humidity on effective nebulized aerosol deposition in different clinical environments.

REFERENCES


