International Journal of Pharmaceutics 463 (2014) 50–61

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

journal homepage: www.elsevier.com/locate/ijpharm

Dynamics of aerosol size during inhalation: Hygroscopic growth of commercial nebulizer formulations☆

Allen E. Haddrell[a,*], James F. Davies[a], Rachael E.H. Miles[a], Jonathan P. Reid[a], Lea Ann Dailey[b], Darragh Murnane[c]

[a] School of Chemistry, University of Bristol, Bristol BS8 1TS, UK
[b] Institute of Pharmaceutical Science, King's College London, London SE1 9NH, UK
[c] University of Hertfordshire, Research Centre in Topical Drug Delivery and Toxicology, Department of Pharmacy, Hatfield AL10 9AB, UK

ARTICLE INFO

Article history:
Received 31 October 2013
Received in revised form
24 December 2013
Accepted 29 December 2013
Available online 6 January 2014

Keywords:
Nebulizer
Hygroscopic growth
Mass flux
Inhalation
Commercial formulations

ABSTRACT

The size of aerosol particles prior to, and during, inhalation influences the site of deposition within the lung. As such, a detailed understanding of the hygroscopic growth of an aerosol during inhalation is necessary to accurately model the deposited dose. In the first part of this study, it is demonstrated that the aerosol produced by a nebulizer, depending on the airflows rates, may experience a (predictable) wide range of relative humidity prior to inhalation and undergo dramatic changes in both size and solute concentration. A series of sensitive single aerosol analysis techniques are then used to make measurements of the relative humidity dependent thermodynamic equilibrium properties of aerosol generated from four common nebulizer formulations. Measurements are also reported of the kinetics of mass transport during the evaporation or condensation of water from the aerosol. Combined, these measurements allow accurate prediction of the temporal response of the aerosol size prior to and during inhalation. Specifically, we compare aerosol composed of pure saline (150 mM sodium chloride solution in ultrapure water) with two commercially available nebulizer products containing relatively low compound doses: Breath®, consisting of a simple salbutamol sulfate solution (5 mg/2.5 mL; 1.7 mM) in saline, and Flixotide® Nebules, consisting of a more complex stabilized fluticasone propionate suspension (0.25 mg/mL; 0.5 mM in saline. A mimic of the commercial product Tobi© (60 mg/mL tobramycin and 2.25 mg/mL NaCl, pH 5.5–6.5) is also studied, which was prepared in house. In all cases, the presence of the pharmaceutical was shown to have a profound effect on the magnitude, and in some cases the rate, of the mass flux of water to and from the aerosol as compared to saline. These findings provide physical chemical evidence supporting observations from human inhalation studies, and suggest that using the growth dynamics of a pure saline aerosol in a lung inhalation model to represent nebulizer formulations may not be representative of the actual behavior of the aerosolized drug solutions.

1. Introduction

For nearly a century, nebulizers have been used to administer medication to the lungs and account for ~13% of all inhaler retail sales in Europe as of 2008 (Lavorini et al., 2011). During this time, the use of the respiratory tract for drug delivery has proven highly successful for treating not only diseases of the lung, such as delivering bronchodilators to treat asthma (Johnson, 1989), but also in the treatment of systemic diseases, such as delivering insulin to treat diabetes (Dubus and Luc, 2003; Watts et al., 2008). In diseases of the lung, and to a lesser extent systemic diseases, the efficacy of the drug can be correlated to the site of drug deposition and delivery (Labiris and Dolovich, 2003). For this reason, the ability to effectively model the eventual site of drug deposition as a function of the composition, size distribution and phase of the nascent aerosol could prove to be advantageous in the design of nebulizer formulations and devices.

Although the importance of the aerodynamic diameter of an aerosol particle in determining the likelihood and site of deposition within the lung is well appreciated (Carvalho et al., 2011), the role of the hygroscopic growth of the aerosol, when produced with a nebulizer, remains a contentious issue. While the hygroscopic growth of fine and ultra-fine aerosol (diameter <1 μm) during inhalation is widely recognized to occur (Asgharian, 2004; Kim et al., 2012), the degree of hygroscopic growth of 'coarse' respirable aerosol (diameter >1 μm) is speculated to be minimal (Dennis, 2009; Finlay, 1998; Finlay and Smaldone, 1998). Given that the aerosol generated by many nebulizers has an aerodynamic size distribution

* Corresponding author. Tel.: +44 0117 331 7388.
E-mail address: a.haddrell@bristol.ac.uk (A.E. Haddrell).

0378-5173/$ – see front matter © 2014 The Authors. Published by Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.ijpharm.2013.12.048

concentrated in the coarse mode (Kallstrom et al., 2008), this assumption suggests that nebulized aerosol will not significantly change size during inhalation, regardless of composition. There are four commonly held reasons that may support the assumption that hygroscopic growth can be neglected. The first reason is that the large number of droplets per unit volume in a nebulized aerosol will stabilize individual particles against hygroscopic size changes. The large evaporating mass of water from the aerosol on nebulization is sufficient to ensure the relative humidity (RH) remains high and, thus, the aerodynamic diameter of all the droplets in the nebulized aerosol will remain approximately constant prior to inhalation (Dennis, 2009).

The second reason hygroscopic aerosol growth is sometimes thought to be minimal during inhalation is that the RH range that a nebulized aerosol experiences during inhalation is considered to be minimal (Dennis, 2009). As with the first reason, this is attributed to the large number of droplets in the aerosol which are believed to sustain the RH at or above 99.5%. When the nebulized airflow encounters any change in RH, the high RH is maintained by a net mass flux of water from the large number of droplets present into the gas phase (Ferron et al., 1997). This, however, is not the case (Prokop et al., 1995), and a series of recent publications have explored this process further (Krajnik et al., 2009; Nerbrink et al., 2003; Zhou et al., 2005). In these studies, the aerodynamic diameter of droplets in a nebulized aerosol is measured as a function of the ambient RH of the air that is mixed with the nebulized aerosol prior to inhalation. The influence of the ambient RH on the size distribution was found to be very much dependent on the pathway of the airflows through the nebulizer. When the only airflow in the system passes through the nebulized region (Fig. 1A(i)), also known as a breath enhanced nebulizer (*e.g.* Pari LC+ (Pari GmbH, Starnberg, Germany)), the RH throughout the system remains at or near saturation (Berg et al., 2007). This results from the ambient air becoming saturated in the nebulization region. As a result, for these types of nebulizers the degree of size change of the aerosol prior to inhalation is expected, and observed (Nerbrink et al., 2003), to be minimal. When the pathway of the ambient airflow instead meets the nebulized airflow at a T-junction (Fig. 1A(ii)) the volume of air and amount of time that the aerosol and ambient air spend mixing are controlled by the breathing rate of the patient and the breathing circuit design. For these systems, it was consistently observed that the RH of the ambient air had a significant and direct effect on the aerodynamic diameter of the aerosol exiting the nebulizer prior to immediate inhalation and the degree of the size change was found to be a function of the ambient RH (Krajnik et al., 2009; Nerbrink et al., 2003; Zhou et al., 2005).

The third reason that hygroscopic growth is sometimes assumed to be minimal during inhalation is the absence of any measurements that allow direct access to the rapid and significant changes in aerosol particle size that can occur at the near-saturation RH and elevated temperature characteristic of the respiratory tract. Specifically, measurements of both the magnitude and rate of condensational growth for aerosols generated from nebulizer formulations have not been possible until recently (Davies et al., 2012b). Without such data, the rate of growth of hygroscopic aerosol has been assumed to be that of pure saline (Xi et al., 2011), precluding any quantitative assessment of the role of hygroscopic growth of coarse nebulized aerosols during inhalation and the dependence on aerosol composition. The approach proposed here is to exploit recently developed single droplet analysis techniques to measure the time dependence of the mass flux to and from an aerosol of known composition as a function of the RH. An improved understanding of the mass flux from a single droplet will enable parameterization of a more accurate condensational growth model compared to previous reports. This new parameterization will then be used to predict the dynamic changes in aerosol size during a variety of hypothetical inhalation scenarios. One example is depicted in Fig. 1B for a constant output jet nebulizer with a T-junction.

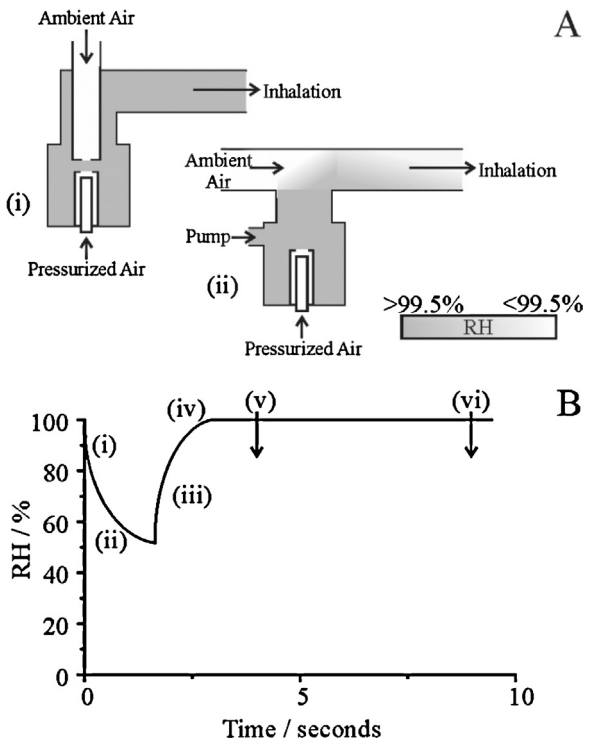

Fig. 1. (A) Two common airflows found in commercially available nebulizers. (B) A hypothetical representation of changes to relative humidity (%) during a wet nebulization inhalation process: (i) represents the aerosol generation process, (ii) denotes aerosol transport phase from the nebulization chamber to the mouthpiece (2 s), (iii) represents onset of aerosol inhalation and (iv) depicts the period aerosol residence in the lower respiratory tract. Points (v) and (vi) represent two different exhalation scenarios: (v) describes a relatively rapid exhalation based on tidal breathing and (vi) represents a hypothetical scenario in which the breath is held for ~10 s prior to exhalation. In both scenarios it is assumed that the RH% will remain at near saturation until exhalation is completed. This representation is based on experimental data from a combination of sources (Ferron, 1994; Martonen et al., 1982; Morrow, 1986).

The fourth reason that hygroscopic growth is often ignored is the assumption that there is insufficient water vapor in the lung to fully supply and sustain the growth of a large number of droplets in a nebulized aerosol. As a result, the amount of growth that is possible within the lungs is considered to be limited. It should be noted that the first, second and fourth issues are connected; either the aerosol is able to buffer the change in ambient/lung RH or not; if it is unable to buffer the change the changes in the RH is then large enough to cause a significant change in the aerodynamic size. In this study, each of these issues will be explored, and the potential importance of hygroscopic growth of nebulized pharmaceuticals in the estimation of dose, both overall and targeted, will be discussed.

The thermodynamic and kinetic factors governing the capacity of aerosols containing active pharmaceutical ingredients in saline solutions to undergo hygroscopic growth during inhalation remain ill-defined (Chan et al., 1994; Martonen et al., 1982). To enhance our understanding of the response of aerosol particle size to rapid changes in humidity for typical nebulizer product formulations, two different commercially available nebulizer formulations are compared with 150 mM sodium chloride (NaCl) solution in ultrapure water (referred to as saline in this study) to determine if the low drug content, either in solution or suspension form, influences hygroscopic growth profiles in nebulized products. Saline is the most commonly used vehicle in nebulizer solutions

and was selected as a reference. The two commercially available nebulizer products chosen to represent low dose formulations were: Breath®, and Flixotide® Nebules.

Secondly, formulations of high dose nebulizer solutions are evaluated to study the impact of high drug, low NaCl content on hygroscopic growth profiles. In this study phase, two tobramycin formulations are compared. The first model formulation (TOB) consists of a 75 mg/mL tobramycin solution (160 mM) with no NaCl content and is loosely based on the commercial product Bramitob© (Chiesi; containing 75 mg/mL tobramycin). The TOB formulation represents a solution in which the drug content is sufficiently high to ensure isotonicity without the need for NaCl as a tonicity regulating excipient. A second model formulation (TOB + NaCl; based on the commercial product, Tobi©, Novartis) contains 60 mg/mL tobramycin (128 mM), 2.25 mg/mL NaCl (39 mM), and a small amount of sulfuric acid to adjust the pH to 5.5–6.5 (final tonicity: 166 mM). Model formulations, rather than their commercial counterparts, are intentionally compared to generate highly accurate models of hygroscopic growth which rely on knowledge of the exact mass composition of each formulation and to better compare the impact of no NaCl vs. small amounts of NaCl on hygroscopic growth of high dose nebulizer solutions.

2. Materials and methods

2.1. Humidity in a nebulized airflow as a function of airflow rate and geometry of mixing

The experimental setup of the apparatus used to measure the RH within a nebulized airflow is shown in Fig. 2A. The ultrasonic nebulizer (Omron, NE-U07) was set to a maximum flow rate throughout. The airflow rates through the system were set using multiple mass flow controllers (MKS Instruments). The flow rate to vacuum was set to the cumulative flow rates or airflows (1) and (2). Once the flow rates of the various airflows were set (ranging between 0 mL/min and 5000 mL/min), time was given for the humidity to reach equilibrium before the measurement was taken. Note that when nebulizers are used to administer medication, time is not given to reach equilibrium. Only deionized water was nebulized in this study to avoid contamination of the probes and subsequent buffering in time response. Thus, the RH measured with this method will be higher than those experienced if the nebulized solution contained a solute, such as saline; the presence of a solute would reduce the amount of water that would be partitioned to the gas phase.

2.2. Nebulizer solutions

A commercially available sterile saline product (Steri-Neb®, 0.9%, w/v) was used. Breath® (salbutamol 5 mg/2.5 mL nebulizer solution) and Flixotide® Nebules (Allen & Hanburys Ltd., Uxbridge, UK; 0.25 mg/mL) were sourced from the manufacturer and used without dilution. While the Breath® formulation contains only salbutamol sulfate and NaCl (equating to a total solids concentration of 1.0% (w/v)), the Flixotide® Nebules formulation also contained undisclosed amounts of polysorbate 20, sorbitanlaurate, monosodium phosphate dehydrate, dibasic phosphate anhydrous, and NaCl in addition to the disclosed drug concentration, precluding calculation of the total solids concentration (%, w/v). Tobramycin powder was purchased from Sigma–Aldrich and dissolved either in water to produce final tobramycin concentrations of 75 mg/mL (equivalent to Bramitob© (Chiesi) without the saline) or in 2.25 mg/mL NaCl solution at a tobramycin concentration of 60 mg/mL (equivalent to Tobi© (Novartis)), before the pH of the solution was reduced to ~6 with dilute sulfuric acid.

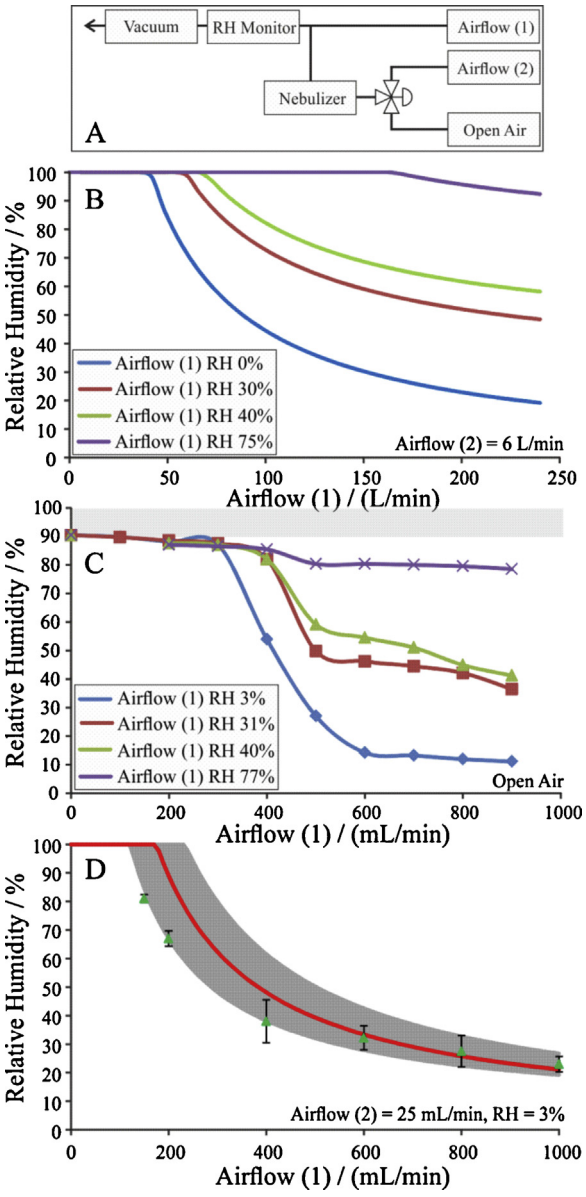

Fig. 2. (A) Experimental setup to measure the RH of an airflow mixed with nebulized water. (B) The predicted limit of the RH in an airflow based solely on the mass of water nebulized. (C) The actual RH in an airflow when no secondary airflow is applied. The light gray bar indicates saturation region of the probe. (D) The actual RH of an airflow when a secondary airflow is controlled and introduced to the nebulizer. Green triangles are the RH measurements, the red line is the expected RH based solely on the mass of water that was nebulized, and the gray area is the error associated with the expected RH from variations in the mass of water nebulized over time. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

2.3. Single aerosol droplet analysis

Three complementary single particle analytical techniques, a double-ring electrodynamic balance (EDB), comparative kinetic EDB (CKEDB), and optical tweezers (OTs), were used in this study. The EDB was used to measure the thermodynamic equilibrium size of droplets across a wide range of RH (from near saturation to dry) for droplets >5 μm in diameter. OTs and the CKEDB were used to investigate the dynamic size changes experienced by droplets due to rapid changes in conditions. Droplets between 3 and 8 μm in diameter were probed in the OT while droplets ranging from 6 μm to ~50 μm were studied in the CKEDB. A model based on the semi-analytical mass flux equations of Kulmala et al. (1993) was used to

interpret and parameterize the kinetic data obtained through both experimental techniques, allowing simulation of aerosol dynamics under physiologically relevant conditions for particle sizes typical of inhalation therapies.

A detailed description of the experimental setup and operation of the EDB has been published previously (Haddrell et al., 2012; Hargreaves et al., 2010) and only a brief overview will be given here. A starting solution/suspension was placed into the reservoir of a droplet-on-demand dispenser (Microfab, MJ-ABP-01with an orifice of 30 μm) that was positioned 4 mm above an induction electrode. The presence of the high voltage induction electrode (+500 V) induced a mirror charge on the droplet resulting in a net negative charge of <50 fC on the droplet as it was ejected from the dispenser. The net charge on the droplet allowed it to be manipulated by the electric field in the EDB, which was generated through application of a 2 $kV_{0\text{-}P}$, 100 Hz signal to two ring electrodes. A DC offset voltage to the AC field was required to maintain the position of the droplet at the null point of the trap (V_{DC}). The mass growth factor of the droplet is reported as the V_{DC} at a given RH divided by V_{DC} of the equivalent dry particle devoid of water at the null point when under dry conditions (RH < 10%). The absolute size of the droplet was measured by taking the angular spacing between fringes in the elastic scattering pattern and a geometrical optics approximation was used to estimate the radius (Haddrell et al., 2012). The V_{DC} and radius were measured continuously as the humidity was varied from high humidity (>85%) to low humidity (<30%) in a stepwise fashion over a 40 h period; the RH was changed in steps of 10% and, at a minimum, an hour was allowed for the whole EDB chamber and particle to equilibrate at each step.

A detailed description of the setup and operation of the CKEDB has been published previously (Davies et al., 2012b; Davies et al., 2013), and only a brief overview will be given here. The CKEDB has many characteristics similar to that of the EDB, most notably the method of generating a droplet with net charge. The primary difference between the two devices is the orientation of the electrodes wherein the CKEDB has two concentric cylindrical electrodes consisting of an outer grounded electrode and an inner electrode with a variable potential of 0–1 $kV_{0\text{-}P}$, (100–500) Hz. This electric field provides a much stronger trapping potential for confinement of the aerosol particle than the ring electrode EDB and allows droplets to be confined within 100 ms from their initial creation at the tip of the dispenser. A trapped droplet was imaged and the size estimated in the same way as the EDB, using the angular variation in the intensity of the elastically scattered light and the geometrical optics approximation. The radius of the droplet was measured with a time resolution of 10 ms until either the particle size reached equilibrium or the particle became too small to remain trapped.

A detailed description of the setup and operation of the optical tweezers has been published previously, and only a brief overview will be given here (Reid et al., 2011). Using an ultrasonic nebulizer (Schill Medical, Aerosonic Travel), a population of saline droplets was dispersed into a trapping cell where a single droplet 3–8 μm in radius was captured in the focal point of a laser beam (532 nm, Opus). The high numerical aperture objective used to form the trap was also used to collect the elastic and inelastically scattered light emitted from the trapped droplet. The diameter of the droplet was measured by comparing the resonant fingerprints of the whispering gallery modes (WGMs) wavelengths in the Raman band with predictions from Mie scattering theory. Through the use of a beam splitter and a mechanical shutter, modulation of the laser light intensity illuminating the droplet was used to induce small changes (~10 mK) in the droplet temperature (Miles et al., 2010). These small changes in temperature led to the sequential evaporation and condensation of water on the droplet, the kinetics of which were recorded by measuring the change in droplet size with sub-nanometer accuracy and with a time resolution of <100 ms. Previously, we have shown that such measurements can allow precise measurements of the kinetics of water transport to or from aerosol particles (Miles et al., 2010).

2.4. Modeling of hygroscopic aerosol growth during inhalation

Experimental data were supported by a number of model simulations to describe both the equilibrium and dynamic response of the aerosol. The equilibrium models, used primarily for saline solution, included both the extended aerosol inorganic model (E-AIM) (Clegg et al., 1998), the aerosol diameter dependent equilibrium model (ADDEM) and the Clegg/Wexler treatment (Clegg et al., 1997, 1998). These treatments were used both to directly compare experimental thermodynamic growth data with theory, as well as to determine the water activity/mass fraction of solute relationships required for use in the mass flux equations, based on the semi-analytical solution to the mass and heat flux equations of Kulmala et al. (1993). The semi-analytic equation was used to simulate the dynamic response of droplets to changes in conditions (*e.g.* a change in RH), such as those imposed on droplets in the OT and CKEDB. The use of this model as a means of simulating data in these experiments has been discussed and verified in detail by Miles et al. (2010) and Davies et al. (2012a,b), and for brevity will not be described here. Although it is not possible at this stage to directly replicate the very high humidities and elevated temperature of the respiratory tract experimentally, the measurements are designed to characterize the equilibrium growth and kinetics of evaporation/condensation over a wide range of conditions, validating the model and providing empirically-derived parameters to achieve robust predictions of the growth dynamics to be expected under the relative humidity condition during inhalation.

3. Results

3.1. Humidity in a nebulizer airflow as a function of nebulizer orientation

In order to predict the RH within a nebulizer airflow as a function of ambient RH and device construction (*e.g.* diameter and location of the T-junction), the amount of water nebulized was first measured as a function of the airflow by weighing the mass of water in the reservoir of the nebulizer before and after a set period of time of nebulization. While the amount of water nebulized as a function of time was found to be dependent on the airflow rate, the overall mass of water in the gas/aerosol phase per unit volume was found to be independent of the airflow rate. The mass of water in a given volume of air was 7.9 ± 2.3 times that expected based on the saturation pressure of water (the majority of which will be in the condensed particle phase) independent of airflow rate.

Estimates of the expected RH of the vapor flow emitted from a standard nebulizer as a function of the secondary airflow rate and RH were based solely on the mass of water emitted by the nebulizer (Fig. 2B). These predictions assume that full mixing occurs and is controlled strictly by the two airflow rates. Given that the breathing rate of a healthy person ranges between 0 and ~20 L/min at any point in time, the predictions in Fig. 2B suggest that the humidity in an inhaled airflow will be saturated regardless of the ambient air RH in flow 2 and the relative flow rates 1 and 2. However, this was found not to be the case when measured (Fig. 2C). When a secondary airflow was added through a T-junction, the humidity of the airflow downstream of the T-junction followed a similar trend as those predicted in Fig. 2B, but at much lower total flow rates with values that are readily achieved during inhalation. This is likely due to the air pressure exerted by the pump rather than the airflow rate, a scenario that is also likely to be applicable on inhalation. Even

though the airflow through commercial constant output nebulizers is typically set to the average breathing rate of 6 L/min, the air pressure at the mouthpiece is very low (measured to be <0.014 psi for the device used in this study). As a result, any incursion of the secondary airflow as a result of this pressure drop will dramatically affect the RH at the point of inhalation, as will the orientation of the airflows in the device (*e.g.* the diameter of the opening of the T-junction). To verify this, the values of all airflows in the system were controlled and the RH was measured. In this situation, the ability to predict the RH at the point of inhalation based solely on the mass of water nebulized was possible (Fig. 2D).

Taken together, the work presented here, coupled with previous studies (Krajnik et al., 2009; Nerbrink et al., 2003; Zhou et al., 2005), clearly demonstrates that aerosol produced from a nebulizer can reach a wide range of RH, even extending below 50%. The extent of the range is dependent on the construction of the nebulizer, the ambient RH, and the airflow rates (ratio of Airflow (1) to Airflow (2), see Fig. 1) through the device. Thus, a detailed understanding of the hygroscopic behavior of nebulized aerosol will be needed to accurately model their deposition in the lung.

3.2. Single aerosol droplet analysis of low dose nebulizer solutions

To maintain the osmotic potential at the surface of the lung during inhalation, nebulizer solutions typically consist of a saline solution with the drug and, in certain cases, other additives. To separate out the role of the saline from that of the drug and additives, the thermodynamic and kinetic hygroscopic properties of saline were first measured. The equilibrium state mass and radial growth factors of saline droplets (the droplet size relative to the size of a dry particle composed entirely of the salt) were determined using the EDB (Fig. 3A and B), and were found to agree with well-established models (E-AIM). The time dependence of the size of a series of droplets evaporating from initially dilute solutions into ~0%, ~50% and ~90% RH in the CKEDB are shown as averaged single time-dependencies in Fig. 3C. The kinetic mass flux equations, incorporating the Clegg/Wexler treatment of water activity and density, were used to predict the temporal variation in droplet size under the experimental conditions. The time to reach the equilibrium droplet size was observed to depend on the relative humidity, and the predicted and measured trends for evaporation and condensation were consistent within the uncertainties of the experiment and simulations. These results are consistent with our previous reported studies for aqueous NaCl solution aerosol (Miles et al., 2010).

In both the double-ring and CKEDBs, the droplets studied were >6 μm in diameter, a size range that does not entirely overlap with the inhalable aerosols produced by jet nebulizers, which produces aerosol droplet diameters that typically span 0.5–10 μm (Kwong et al., 2000) with median values usually ranging from 2 to 7 μm, depending on nebulizer type and formulation properties (Mccallion et al., 1995). Of note, is that nebulized aerosol droplets are frequently undersized due to evaporation under the ambient environmental conditions at which measurements are typically performed. Further, the measurements made by the EDBs only access RHs as high as ~90%, again not directly representative of the RH observed in the respiratory tract. OTs were therefore used to validate the model treatment for the condensational growth kinetics of saline aerosol droplets in a size regime closer to that expected from a medical nebulizer (<8 μm diameter) and at an RH closer to that representative of the respiratory tract (>95% RH). Following the procedure adopted in our previous work (Miles et al., 2010), the return of the droplet to an equilibrium size by condensational growth was monitored following a transient perturbation in vapor pressure induced by a small change in temperature. An example of such a transient growth event at very high RH is shown in Fig. 3D. The same mass flux equations with the same parameterizations for water activity/mass fraction of solute were used for both size regimes and over the full range of RH conditions, confirming that the model is capable of predicting the dynamic response of droplets across broad size and RH ranges, and for both evaporation and condensation. Furthermore, the importance of including a correction for the slow heat flux by collisions with gas phase nitrogen/oxygen molecules is clear. Although the actual growth of the droplet was low (Fig. 3D), the consequence of neglecting this correction is evident from the under-prediction of the time to reach equilibrium if heat transport is ignored. Failure to correct for heat flux underestimated the time to reach equilibrium by ~2 s, thereby demonstrating the need for accurate measurements of growth rates to support deposition modeling, rather than assuming equilibrium conditions. Indeed, for the droplet sizes typical of inhalation, the rate of mass flux is largely controlled by the flux of heat back into the gas phase rather than the diffusional transport of mass. Neglecting the limitations imposed on the growth rate by the slow heat conduction away from the droplet leads to a significant over-prediction of the growth rate.

Most commercial nebulizer formulations of salbutamol sulfate are simple two component systems containing saline and the soluble drug. The thermodynamic hygroscopic properties of the Breath® formulation, obtained in the same manner as pure saline, are shown in Fig. 4A and B, reporting both the relative mass and radial growth with RH. The dissolved solids content of the formulation was calculated to be 1.0% (w/w), of which NaCl comprised 81% (w/w) and salbutamol sulfate comprised only 19% (w/w). It is therefore not surprising to see that the equilibrium hygroscopic growth curve (*i.e.* the relative radial growth factors) was similar in form (distinct efflorescence and deliquescence points) to that of saline but with a significant suppression in the growth due to the presence of less hygroscopic salbutamol sulfate (Fig. 4A). This is at odds with previous studies which have suggested that the behavior of a similar compound, isoproterenol hydrochloride, was identical to NaCl aerosols (Martonen et al., 1982). The CKEDB measurements of the time-dependence in droplet size during evaporation from the droplet as a function of humidity are shown in Fig. 4C. A comparison of Figs. 3C and 4C shows a minimal difference between evaporation profiles for RH values 50% and lower, indicating no significant impact of the drug mass on the evaporation kinetics and no alteration of phase behavior, such as would be observed if a glass or gel were formed by the organic components (Bones et al., 2012).

The thermodynamic properties of Flixotide® Nebules formulation are shown in Fig. 4D and E. Like Breath®, the Flixotide® Nebules formulation had distinct efflorescence and deliquescence points, likely due to the NaCl in the formulation, with a slight reduction in hygroscopic growth factor due to the presence of non-hygroscopic components. Based on the magnitude of reduction in mass growth factor across all RH, the weight percent (w/w) of NaCl out of the total mass of dissolved solids may be estimated to be below 50%, indicating a significant proportion of the solute mass likely consists of the additional excipients. Fluticasone propionate is insoluble in water. Thus, the Flixotide® Nebules formulation consists of a multi-component system containing not only saline and the active ingredient, but also an undisclosed amount of several other excipients used to stabilize the active ingredient in the suspension. This missing information prevents the calculation of the exact mass fractions present in the formulation, as well as the relative contributions to hygroscopicity of each component. CKEDB measurements of the evaporation kinetics upon injecting droplets into various humidities are shown in Fig. 4F. As with the salbutamol sulfate formulation, droplets from Flixotide® Nebules showed behavior similar to saline droplets, the evaporation rate was limited by gas phase diffusional transport of water away from the droplet and heat to the droplet surface.

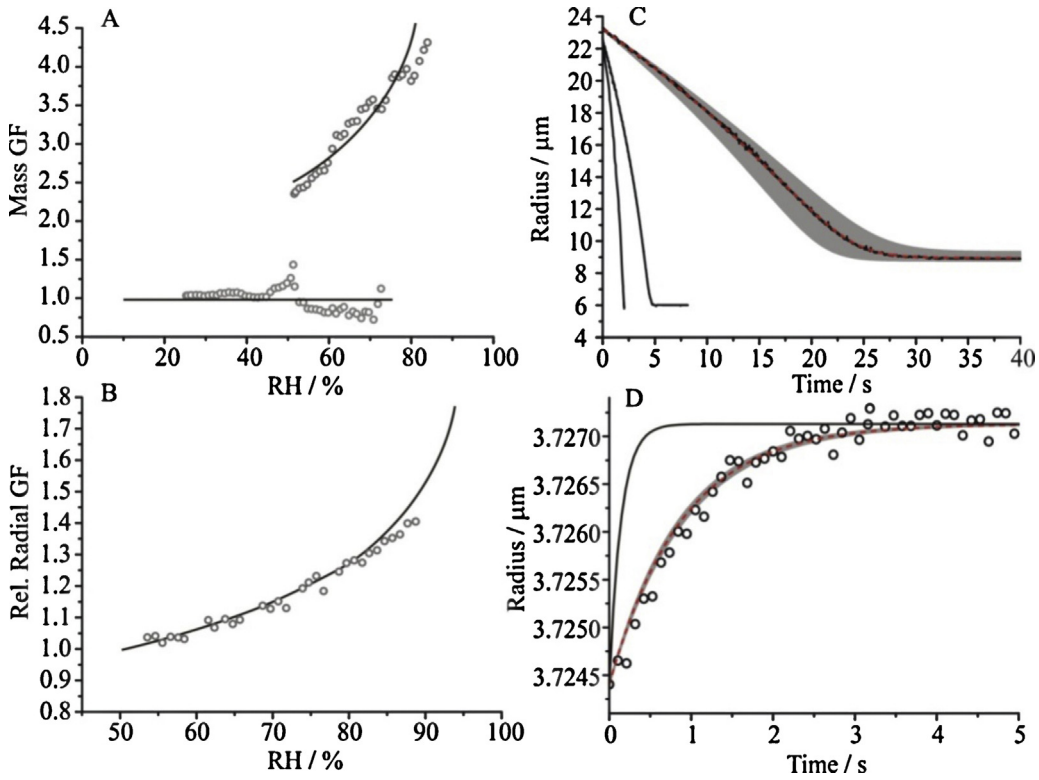

Fig. 3. Equilibrium state mass (A) and radial (B) growth factors of saline as a function of RH, as measured with the EDB. The deliquescence/efflorescence cycle is clear in (A), while (B) reports measurements of the relative size change for solution droplets at RHs above the efflorescence RH. (C) The time-dependence of the size of saline aerosol droplets injected into an air flow with relative humidity of 90%(right curve), 50% (mid curve) and <10% (dry air; left curve), as measured with the CKEDB. The red line indicates the model prediction while the gray bar indicates error associated with the model due to the uncertainty in the RH (±1%). At 50% and 90% RH, the evaporating droplets reach an equilibrium size. (D) The time-dependence of the radius of a saline droplet during condensational growth from an initial state to a final equilibrium state, as measured with OT. The experimental data are indicated by circles. Model predictions are indicated by lines, with the influence of latent heat included(red) and not included (black) for comparison. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

The semi-analytical treatment for calculating the mass flux during evaporation or condensation at any moment in time requires knowledge of the variation of the water activity (equivalent to the vapor pressure of water) with mass-fraction of solute. This is required in order to calculate the concentration gradient in the gas phase. The concentration gradient can be calculated from knowledge of the gas phase RH and droplet water activity, derived either from thermodynamic models or from equilibrium hygroscopicity measurements (*i.e.* the radial growth factors from Figs. 3B and 4B and E). From the semi-analytic treatment, the instantaneous mass flux is calculated and, thus, the mass loss/gain over a short time step. The new droplet radius at the end of this time step must then be calculated to once again use the radial growth factor data to estimate the new diffusional gradient in the gas phase and the mass loss in the next time step. This requires knowledge of the density of the aerosol droplet with varying composition across the entire range of water activity (*i.e.* RH%). Solution densities cannot be measured over the full RH range from bulk phase measurements (solute concentrations are typically well above the solubility limits below 80% RH) and must instead be determined from aerosol measurements. To estimate the density of a solution, it is necessary to know both the wet and dry diameters of the aerosol as well as the mass of the solution droplet relative to the dry particle. When the fraction of NaCl in the total solids mass (%w/w) is high, the aerosol has a defined efflorescence point that results in the formation of a non-spherical particle whose diameter cannot be measured by light scattering. Thus, for both commercially available salbutamol sulfate and fluticasone propionate formulations, the low concentration of the drug in the saline solution affects the radial growth factor but not the efflorescence point, and the absolute dry diameter and thus solution density cannot be determined. This precludes a fully robust simulation of the evaporation kinetics, a potentially significant limitation when examining evaporation to low RH and preventing direct comparison with a model in Fig. 4C and E. The evaporation curves are, however, qualitatively very similar and occur over similar timescales to the evaporation of saline droplets. For the extremely high RHs (>95%) for which the simulations are applied to predict growth in the respiratory tract later in the paper, assuming that the density is equal to that of the saline solution (approximately the same as water) introduces only negligible error.

3.3. Single aerosol droplet analysis of high dose nebulizer solutions

Tobramycin solutions were chosen as an appropriate model system to investigate the impact of high dose drug solutions and low NaCl content on hygroscopic growth profiles. As mentioned above, in order to accurately model aerosol dynamics, the absolute chemical composition of the starting formulation must be known to a high degree of certainty. Although the composition of the commercial formulations of Tobi© is readily available, an issue arises when the pH of the commercially available solution is pH adjusted. This results in a slight change in the concentration of all the solutes in the starting formulation. As a result, the ability to accurately model, to a very high degree of certainty, the rate and magnitude of mass flux derived from the hygroscopic growth measurements is not possible. To address this, TOB and TOB + NaCl formulations were prepared from the bulk, where the precise chemical composition of the starting formulation was known.

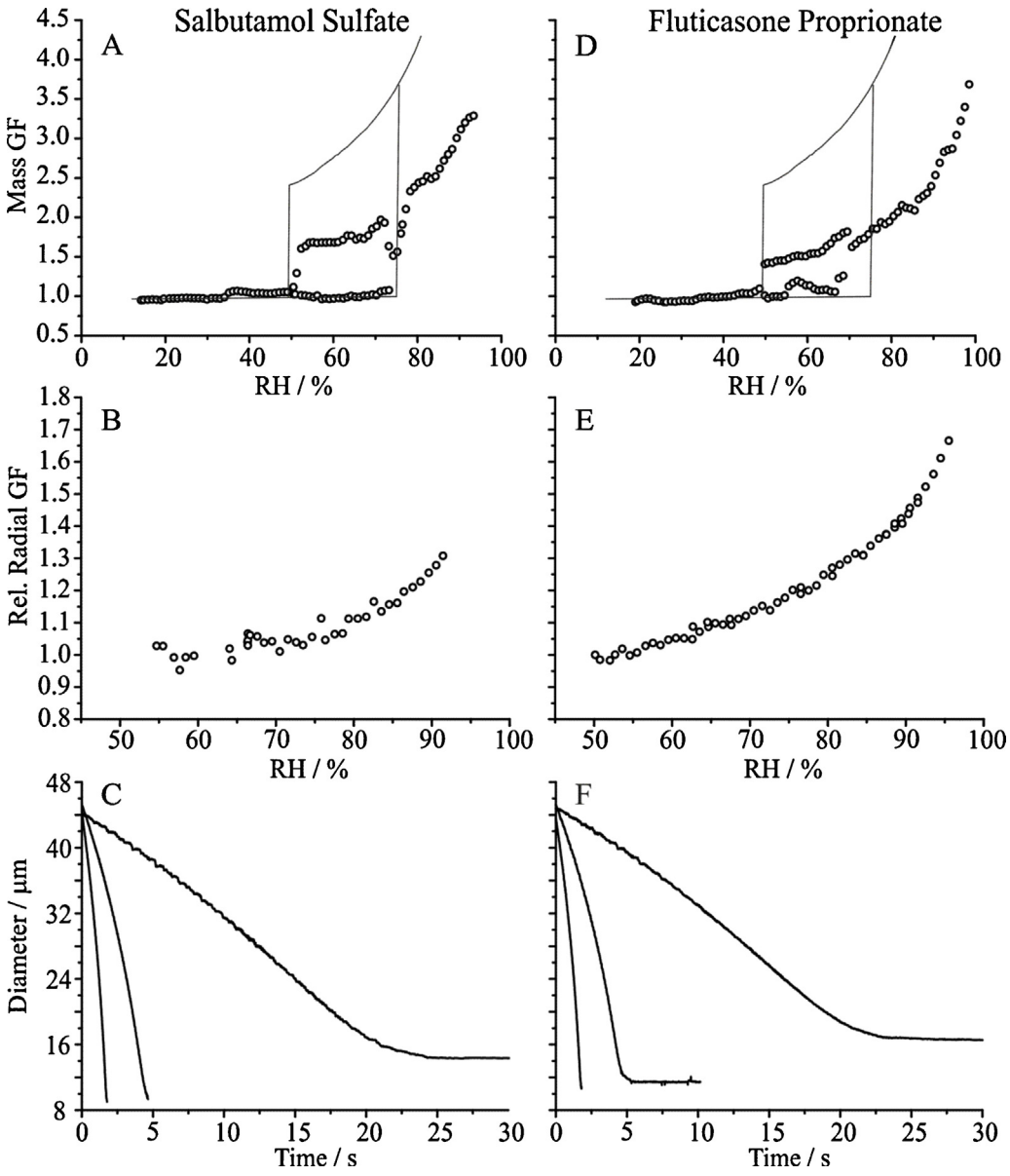

Fig. 4. Evaluation of two low dose commercial nebulizer preparations: salbutamol sulfate (left panel) and fluticasone propionate (right panel). Mass (A and D) and radial (B and E) growth factors of aerosol droplets as a function of RH, as measured with the double ring EDB. The solid lines in A and D indicate the mass growth curve for pure saline. (C and F) The time dependence of the size of low dose drug aerosol droplets injected into an air flow with relative humidity of 90% (right curve), 50% (mid curve) and <10% (dry air; left curve) as a function of time (measured with the CKEDB). OT measurements of condensational growth were not performed, as the lack of disclosed information regarding dissolved excipient mass in these commercial preparations prevented computation of the growth model.

The mass and radial growth factors as a function of RH are shown in Fig. 5A and B for TOB and Fig. 5D and E for TOB + NaCl. Droplets of these formulations were injected into three different relative humidities and the mass flux measured, as depicted in Fig. 5C for TOB and 5F for TOB + NaCl. Similar to the three previous formulations, the evaporation kinetics were limited by gas phase diffusion for TOB across all RHs. Kinetic limitations in the rate of evaporation was observed when TOB + NaCl was injected into an airflow of 50% RH, as evidenced by the continual loss of water from ~4 to ~8 s. This suggests the formation of an amorphous state or the presence of surface active species.

Unlike the salbutamol sulfate and fluticasone propionate formulations, deliquescence and efflorescence points were not observed for the TOB and TOB + NaCl formulations. Thus, it was possible to collect accurate radial data down to low RH for both (Fig. 5B and E), to determine the dry radii and, hence, growth factor across a wide range of water activities (Fig. 6A and C). From such a complete picture of the thermodynamic properties and densities, the kinetics of water evaporation and condensation using the semi-analytic treatment can be fully predicted to a high degree of certainty (Fig. 6B and D). Remarkably good agreement with the experimental measurements was observed for the relative humidity region in which growth factors were determined, well within the uncertainty associated with the relative humidity, which was by far the largest uncertainty present in the simulation. Again, evidence of the formation of the amorphous state is observed for the TOB + NaCl formulation in Fig. 6D, where the experimental data deviates from the model prediction between ~4 and ~8 s.

3.4. Modeling of hygroscopic aerosol growth during inhalation using experimentally-determined growth factors

The ability to use the measured relative mass, radial growth factor, and density of the aerosol at equilibrium as a function of

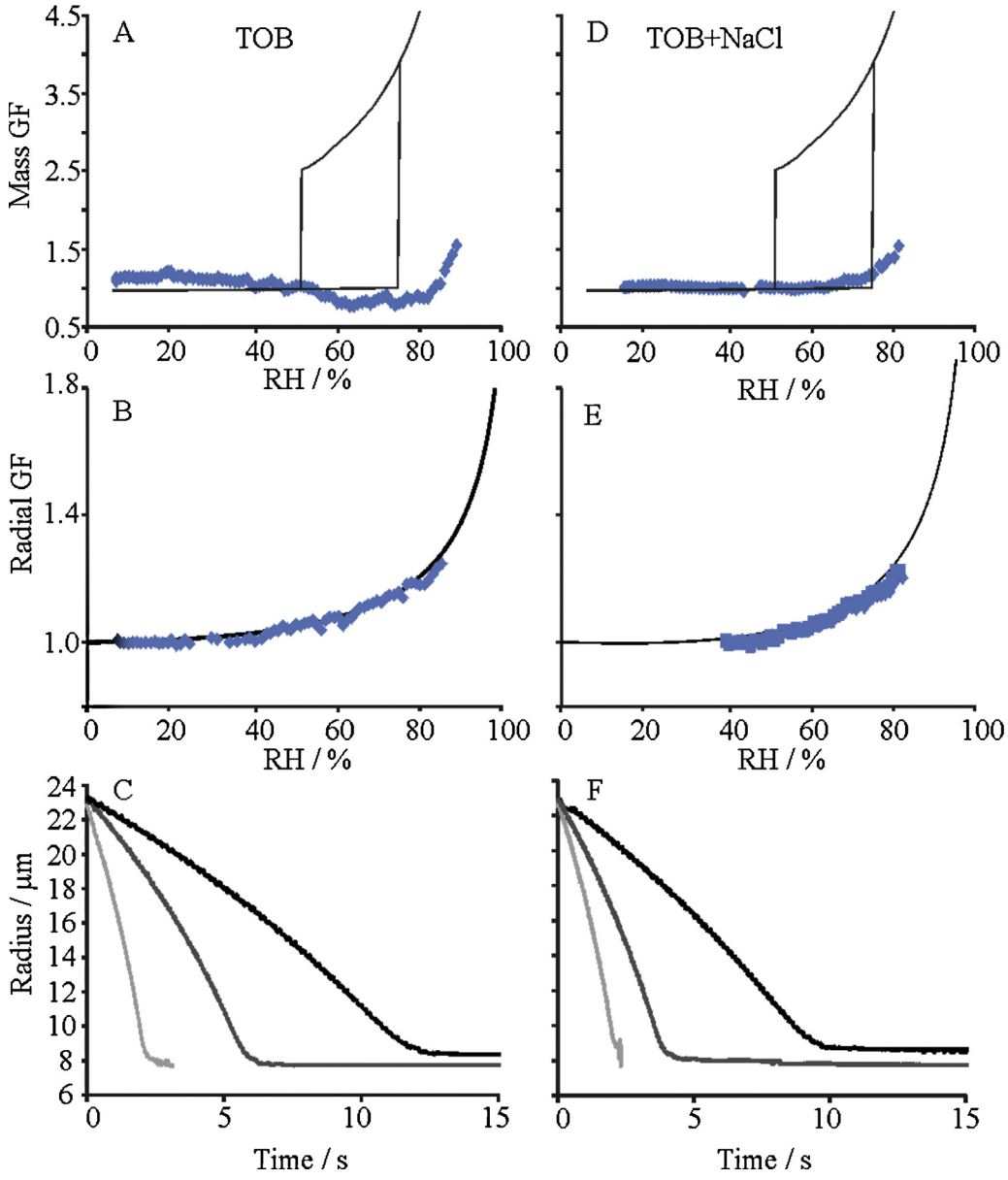

Fig. 5. Mass (A and D) and radial (B and E) growth factors of TOB and TOB + NaCl aerosol droplets as a function of RH, as measured with the double ring EDB. The solid line in (A and D) indicates the mass growth curve for pure saline. The solid line in figures B and E are extrapolations of the radial growth factor. (C and F) The time dependence of the aerosol droplets injected into an air flow with relative humidity of 90% (black curves), 50% (dark gray curves) and <10% (dry air; light gray curves) as a function of time (measured with a CKEDB).

water activity and composition, and to predict the independent experimental mass flux measurements collected with the CKEDB demonstrates the high degree of understanding achieved for the high dose solution systems. Thus, accurate predictions can be made of mass flux in dynamic environments, such as that experienced by an aerosol during inhalation. To highlight the potential use of these mass flux simulations, the kinetics of aerosol evaporation/condensation were compared for the TOB, TOB + NaCl and saline aerosols (Fig. 7) in response to very subtle increases in RH at high humidity (*i.e.* elevation from 98 to 99.5% RH). These conditions might reflect a hypothetical scenario in which the humidity within a given nebulizer remains nearly saturated at ~98% RH and the generated aerosol travels rapidly through the device prior to entering the higher humidity environment of the respiratory tract. Fig. 7A depicts the extent of aerosol size change when a new droplet of saline (a_w of 0.995) with a diameter ~5 μm is generated and held at 98% RH for 5 s (undergoing evaporation) prior to rapid elevation of the RH to 99.5%, whereby condensational growth commences. Fig. 7B shows the same aerosol droplet injected into and held at 98% RH for 1s prior to elevation of RH to 99.5%. In this case, evaporation time is significantly decreased and thus the magnitude of condensational growth in 99.5% RH is reduced. Fig. 7C depicts the same scenario, except that the initial period of droplet residence at 98% is reduced to 0.5 s, resulting in a further significant decrease in droplet evaporation and a swift achievement of equilibrium size after condensation growth at 99.5% RH. Note that for a saline droplet with a starting diameter of 5 μm, its size is never the same as the tobramycin containing droplets at any point in time; this further demonstrates the problems associated with indiscriminant use of saline as a model system to predict the hygroscopic growth of nebulized aerosols during inhalation, especially when formulations contain low or no NaCl content. As a case in point, the TOB + NaCl formulation studied here behaves hygroscopically much more like the TOB formulation than the saline solution.

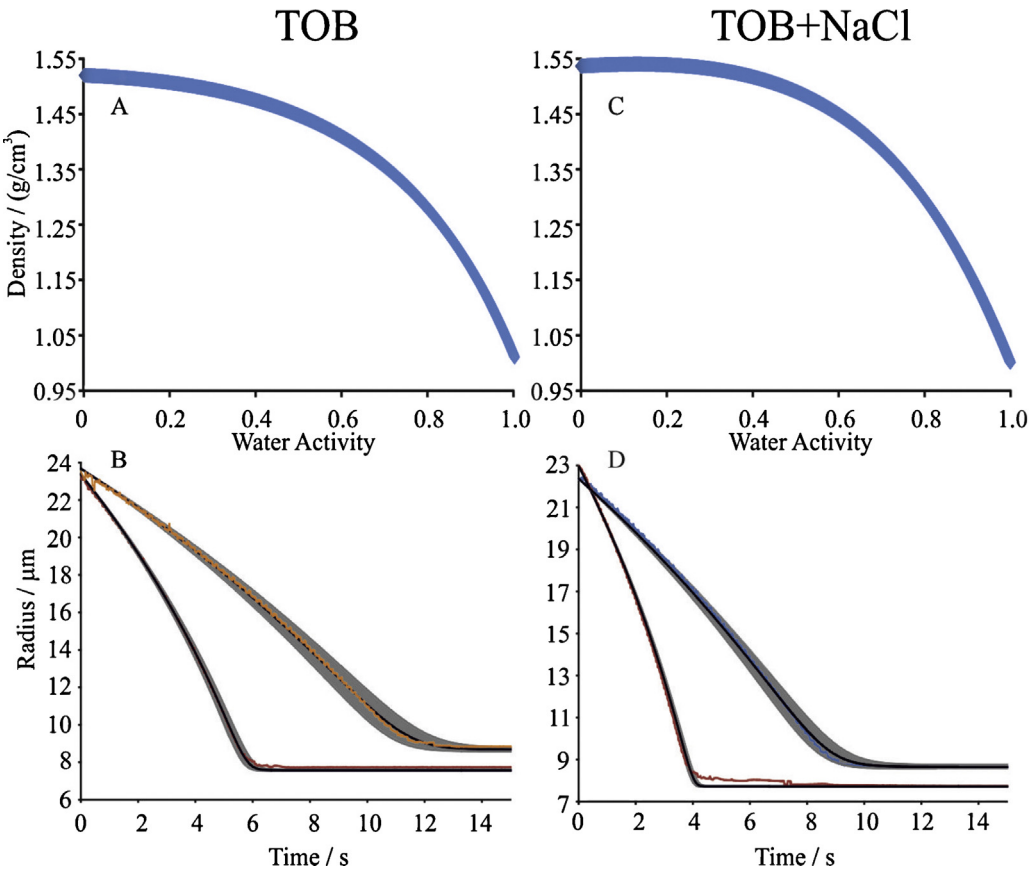

Fig. 6. Density as a function of RH (A, C) of TOB and TOB + NaCl, the changes to the radius of this aerosol following its injection into an air flow with a RH of ~80%and ~55% were modeled (B, D). In B and D, the black line indicates the model prediction while the gray area indicates the error of the model resulting from the uncertainties associated with the RH (±1%).

It has previously been suggested that coarse droplets (>1 μm) do not reach equilibrium during lung residence times of 5 s (Broday and Georgopoulos, 2001). However, the availability of empirically-derived growth factors from the current work shows that equilibrium size may actually occur during relevant time periods for coarse aerosols although it depends on the RH trajectory experienced by the aerosol and on the 'coupling' of total aerosol surface area for condensation to water mass available in the lungs to condense. For the tobramycin solution droplets, less evaporation is predicted to occur over the same time frame at 98% RH compared to the saline droplets. Further, condensational growth occurring at 99.5% was attenuated for both TOB and TOB + NaCl. The ability to accurately predict changes to droplet sizes in response to both subtle and profound changes in humidity may have the potential to significantly improve *in silico* models of aerosol deposition, such as computational fluid dynamic models (Forbes et al., 2011). Also, a greater understanding of how the formulation of high dose non-hygroscopic drug compounds or NaCl content influences the changes to droplet size during nebulization and inhalation, may inform rational formulation strategies for nebulizer products in the future (Haddrell et al., 2013).

Further evidence of the deficiencies of non-discriminant application of saline growth rates to all nebulizer formulations to approximate dynamic changes to droplet size is highlighted in Fig. 8A. This simulation depicts the simplified scenario of what would happen to three equally sized droplets (2.0 μm diameter at an RH of 50% prior to inhalation) containing the formulations saline, TOB and TOB + NaCl. Note that for the aerosol droplets to have an identical diameter at a given humidity, the weight percent of NaCl in the solute of the saline droplet will always be lower due to the highly hygroscopic nature of NaCl. The kinetic growth profiles of these identically sized droplets as they enter an area of high humidity (*i.e.* the mouth and respiratory tract) demonstrate that the saline droplet has a larger magnitude of mass flux than the less hygroscopic tobramycin solutions, *i.e.* saline droplets will experience greater hygroscopic growth in the respiratory tract compared to non-hygroscopic formulations, such as the high dose TOB and TOB + NaCl solutions. The disparity grows as the time spent in the respiratory system increases (Fig. 8B). Along with Fig. 7, these data demonstrate that general predictions of drug deposition in the lung based on a saline model are questionable. Further, non-hygroscopic formulations may have the potential for greater peripheral lung deposition due to their relative lack of hygroscopic growth in the respiratory tract.

4. Discussion

Aerosol growth during inhalation is dependent on the availability of water vapor in the lung, and may be limited by the high particle count in a nebulized aerosol. Beyond the sheer number of droplets within the airflow, the composition, initial size, and RH the droplets experience immediately prior to inhalation will also play a major role in reducing aerosol growth during inhalation. The threshold number concentration of saline particles present in a nebulized aerosol whose hygroscopic growth will be limited by the availability of water vapor in the lung as a function of the aerosol median diameter immediately prior to inhalation is estimated in Fig. 9. These estimations are based on the total mass of water vapor in the lung (Nerbrink et al., 2003) and the total mass of water required for the growth of the entire population of saline droplets of

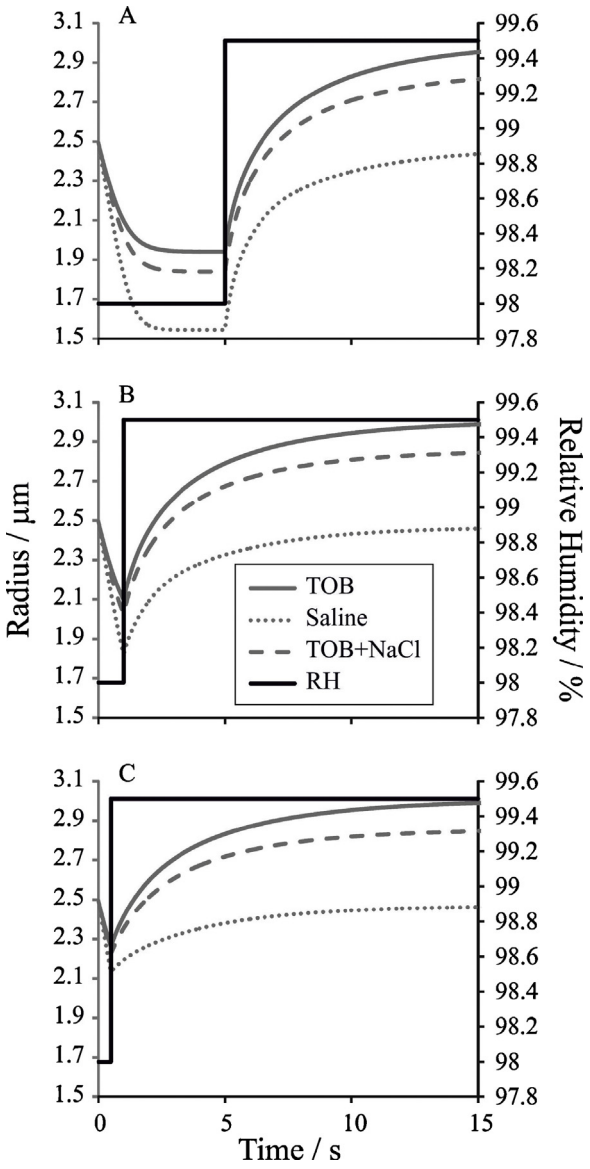

Fig. 7. Model prediction of the time-dependence in the radius of an aerosol consisting of TOB, TOB + NaCl, and saline in response to very subtle changes in RH (thick black line) with all droplets initially 2.5 µm in radius when generated. (A) Depicts a hypothetical scenario in which the nebulizer RH is 98% and the droplet residence time in the nebulizer is 5 s after which inhalation increases the RH to 99.5%. Exhalation is not depicted. (B and C) depict the same scenario as described above, but the droplet residence time in the nebulizer at 98% RH has been decreased to 1 and 0.5 s, respectively.

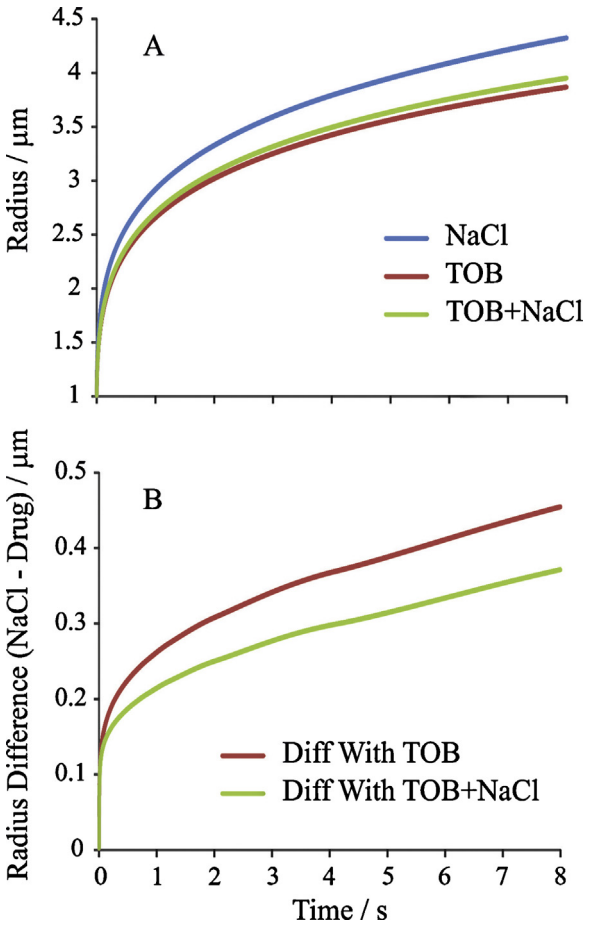

Fig. 8. (A) Growth of pure saline, TOB and TOB + NaCl droplets with an identical starting radius at 1 µm and equal water activity (equivalent to equilibrium at 50% RH) injected into an atmosphere of 99.5% RH. (B) The difference between the radii of the saline droplet and that of a droplet with the same starting radius made up of the drug shown in (A).

a given starting size. Generally speaking, when particle concentrations are above the line, water availability will determine the rate and magnitude of mass flux during inhalation; when particle concentrations are below the line there is theoretically enough water in the vapor phase in the lung to sustain the degree of aerosol growth during inhalation expected from the solution thermodynamics.

The droplet number concentration produced by a series of commercially available nebulizers was calculated based on the data reported by Reisner et al. (2001) and is also shown in Fig. 9. For all of the nebulizers shown, there is expected to be sufficient water to account for the growth of the aerosol in the nebulized airflow when the RH prior to inhalation is at 98%. This results from less water being required for the hygroscopic growth of the saline present at an RH of 98%. This means that the predictions of aerosol growth shown in Fig. 7 are applicable to commercially available nebulizers

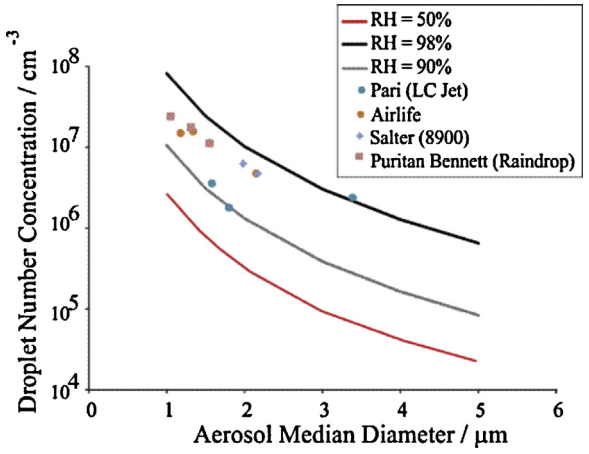

Fig. 9. The concentration of inhaled droplets with a given aerosol median diameter whose growth during inhalation will be limited by the availability of water vapor present in the lung. When the aerosol concentration is above the appropriate line the aerosol growth will be controlled by the available mass of water in the gas phase. When the aerosol concentration is below the line the aerosol growth should be unaffected. Line color indicates the RH the population of the aerosol was equilibrated with immediately prior to inhalation. The data points are the number concentration and aerosol median diameter for a series of commercial nebulizers, and were estimated from data published previously (Reisner et al., 2001).

and, as a result, indiscriminant application of saline growth profiles to estimate the behavior of non-hygroscopic solutions, such as the tobramycin formulations studied here, will lead to over-prediction of droplet growth in inhalation models.

In the estimation presented in Fig. 9, the assumption is made that the mass of water in the vapor phase is finite. In the lung however, with its large surface area, warm temperature and moist surfaces, any reduction in the mass of water in the vapor phase through uptake by an aerosol may be replenished by the lung itself. For this reason, the estimations made in Fig. 9 are likely an over-estimation of the importance of droplet count on limiting aerosol growth, and in reality all of the lines should likely be moved up.

How the magnitude of overall mass flux of an aerosol during inhalation results in changes in the total and regional doses have been modeled previously (Asgharian, 2004; Mitsakou et al., 2005). Within these models, hygroscopic growth was found to have a large effect where the minimum in the total deposition fraction of hygroscopic aerosol range between 500 and 800 nm while the minimum for the hydrophobic aerosol centered around 100 nm. Given that the median aerodynamic diameter of nebulized therapeutic aerosols is around 2 μm (Reisner et al., 2001), only a slight difference in total deposited dose would be expected. However, the pattern of deposition would be expected to differ significantly, wherein aerosols with a high saline content (*i.e.* low dose formulations) may show a higher proportion of upper airway deposition, while aerosols with a lower hygroscopicity (*i.e.* high dose formulations with little to no NaCl) may exhibit greater peripheral deposition. The degree to which this shift in deposition site occurs as a result of the size change measured in this study (Fig. 8) is unclear at this time, and requires detailed modelling to explore further.

5. Conclusions

The ability to accurately model the aerodynamic diameter of an aerosol following its transition between airflows of various RH has been demonstrated. This then allows the kinetics of aerosol evaporation/condensation profiles to be modeled for a variety of pharmaceutically relevant inhalation scenarios, during which aerosol residence in the device, for example, can vary dramatically. With the increasing variety of nebulization formats and device designs (*e.g.* traditional jet nebulizers connected either through tubing to face masks or directly to the mouthpiece, aerosol delivery during mechanical ventilation, discrete hand-held nebulizers, breath-actuated devices and slow mist formats (Watts et al., 2008)) this model is of great relevance.

Through comparison of pharmaceutically relevant formulations, this study has demonstrated that the inclusion of high dose, non-hygroscopic drugs or decrease in NaCl additives can significantly change the equilibrium hygroscopic response of the aerosol and can alter droplet evaporation and growth kinetics during an inhalation event. Thus, overall, the model developed in this study may be very valuable in enhancing *in silico* models of pharmaceutical aerosol deposition in the lung, especially if combined with more accurate patient inspiratory breathing profiles. Further, the results of this study deepen our understanding of dynamic changes to droplet size during the nebulization process itself and are adaptable to a wide range of nebulization formats. Finally, a greater understanding of how formulation parameters influence the kinetics of droplet evaporation/condensation during a real time inhalation cycle will inform rational formulation strategies for nebulizer solutions in the future. Adaptation of the EDB and OT technology to measure dynamic changes in propellant-containing pressurized metered dose inhaler formulations as well as dry powders formulations to explore the impact of real time humidity changes on these systems is currently under way. The results of these future studies promise to enhance our understanding of, as well as enable us to more accurately predict and manipulate, drug deposition of pharmaceutical aerosols in the lung.

Acknowledgments

We thank the Elizabeth Blackwell Institute (EBI) for financial support through the EBI Early Career Research Fellowship awarded to AEH, and the EPSRC for financial support through a Leadership Fellowship awarded to JPR (grant reference EP/G007713/1).

References

Asgharian, B., 2004. A model of deposition of hygroscopic particles in the human lung. Aerosol Sci. Tech. 38, 938–947.
Berg, E., Svensson, J.O., Asking, L., 2007. Determination of nebulizer droplet size distribution: a method based on impactor refrigeration. J. Aerosol Med. 20, 97–104.
Bones, D.L., Reid, J.P., Lienhard, D.M., Krieger, U.K., 2012. Comparing the mechanism of water condensation and evaporation in glassy aerosol. Proc. Natl. Acad. Sci. U.S.A. 109, 11613–11618.
Broday, D.M., Georgopoulos, P.G., 2001. Growth and deposition of hygroscopic particulate matter in the human lungs. Aerosol Sci. Tech. 34, 144–159.
Carvalho, T.C., Peters, J.I., Williams, R.O., 2011. Influence of particle size on regional lung deposition – what evidence is there? Int. J. Pharm. 406, 1–10.
Chan, H.K., Phipps, P.R., Gonda, I., Cook, P., Fulton, R., Young, I., Bautovich, G., 1994. Regional deposition of nebulized hypodense nonisotonic solutions in the human respiratory-tract. Eur. Respir. J. 7, 1483–1489.
Clegg, S.L., Brimblecombe, P., Liang, Z., Chan, C.K., 1997. Thermodynamic properties of aqueous aerosols to high supersaturation. 2. A model of the system Na^+–Cl–NO_3^-–SO_4^{2-}–H_2O at 298.15 K. Aerosol Sci. Tech. 27, 345–366.
Clegg, S.L., Brimblecombe, P., Wexler, A.S., 1998. Thermodynamic model of the system H^+–NH_4^+–Na^+–SO_4^{2-}–NB_3^-–Cl^-–H_2O at 298.15 K. J. Phys. Chem. A 102, 2155–2171.
Davies, J.F., Haddrell, A.E., Miles, R.E., Bull, C.R., Reid, J.P., 2012a. Bulk, surface, and gas-phase limited water transport in aerosol. J. Phys. Chem. A 116, 10987–10998.
Davies, J.F., Haddrell, A.E., Reid, J.P., 2012b. Time-resolved measurements of the evaporation of volatile components from single aerosol droplets. Aerosol Sci. Tech. 46, 666–677.
Davies, J.F., Haddrell, A.E., Rickards, A.M., Reid, J.P., 2013. Simultaneous analysis of the equilibrium hygroscopicity and water transport kinetics of liquid aerosol. Anal. Chem. 85 (12), 5819–5826.
Dennis, J., 2009. Evolution of Evaporative Understanding within Nebulizer Standards. J. Aerosol Med. Pulmonary Drug Deliv. 22, 5–8.
Dubus, J.C., Luc, C., 2003. Inhalation in children: what's new? Revue Francaise D Allergologie Et D Immunologie Clinique 43, 446–449.
Ferron, G.A., 1994. Aerosol properties and lung deposition. Eur. Respir. J. 7, 1392–1394.
Ferron, G.A., Roth, C., Busch, B., Karg, E., 1997. Estimation of the size distribution of aerosols produced by jet nebulizers as a function of time. J. Aerosol Sci. 28, 805–819.
Finlay, W.H., 1998. Estimating the type of hygroscopic behavior exhibited by aqueous droplets. J. Aerosol Med. 11 (4), 221–229.
Finlay, W.H., Smaldone, G.C., 1998. Hygroscopic behavior of nebulized aerosols: not as important as we thought? J. Aerosol Med. 11, 193–195.
Forbes, B., Asgharian, B., Dailey, L.A., Ferguson, D., Gerde, P., Gumbleton, M., Gustavsson, L., Hardy, C., Hassall, D., Jones, R., Lock, R., Maas, J., McGovern, T., Pitcairn, G.R., Somers, G., Wolff, R.K., 2011. Challenges in inhaled product development and opportunities for open innovation. Adv. Drug Deliv. Rev. 63, 69–87.
Haddrell, A.E., Davies, J.F., Yabushita, A., Reid, J.P., 2012. Accounting for changes in particle charge, dry mass and composition occurring during studies of single levitated particles. J. Phys. Chem. A 116, 9941–9953.
Haddrell, A.E., Hargreaves, G., Davies, J.F., Reid, J.P., 2013. Control over hygroscopic growth of saline aqueous aerosol using Pluronic polymer additives. Int. J. Pharm. 443, 183–192.
Hargreaves, G., Kwamena, N.O.A., Zhang, Y.H., Butler, J.R., Rushworth, S., Clegg, S.L., Reid, J.P., 2010. Measurements of the equilibrium size of supersaturated aqueous sodium chloride droplets at low relative humidity using aerosol optical tweezers and an electrodynamic balance. J. Phys. Chem. A 114, 1806–1815.
Johnson, C.E., 1989. Principles of nebulizer-delivered drug therapy for asthma. Am. J. Hosp. Pharm. 46, 1845–1855.
Kallstrom, H., Colice, E., MacIntyre, D., Diette, S., Rubin, B.K., 2008. Aerosol delivery devices in the treatment of asthma – discussion. Respir. Care 53, 723–725.
Kim, J.W., Xi, J., Si, X.A., 2012. Dynamic growth and deposition of hygroscopic aerosols in the nasal airway of a 5-year-old child. Int. J. Numer. Meth. Biomed. Eng. 29 (1), 17–39.
Krajnik, M., Podolec, Z., Zylicz, Z., Jassem, E., 2009. Air humidity may influence the aerosol distribution of normal saline administered by closed or vented nebulizers operated continuously or dosimetrically. J. Aerosol Med. Pulmonary Drug Deliv. 22, 29–34.
Kulmala, M., Vesala, T., Wagner, P.E., 1993. An analytical expression for the rate of binary condensational particle growth. Proc. R. Soc. A: Math. Phys. Eng. Sci. 441, 589–605.
Kwong, W.T.J., Ho, S.L., Coates, A.L., 2000. Comparison of nebulized particle size distribution with Malvern laser diffraction analyzer versus Andersen Cascade

Impactor and low-flow marple personal cascade impactor. J. Aerosol Med. 13, 303–314.
Labiris, N.R., Dolovich, M.B., 2003. Pulmonary drug delivery. Part I: physiological factors affecting therapeutic effectiveness of aerosolized medications. Br. J. Clin. Pharmacol. 56, 588–599.
Lavorini, F., Corrigan, C.J., Barnes, P.J., Dekhuijzen, P.R.N., Levy, M.L., Pedersen, S., Roche, N., Vincken, W., Crompton, G.K., Improvemen, A.D.M., 2011. Retail sales of inhalation devices in European countries: So much for a global policy. Respir. Med. 105, 1099–1103.
Martonen, T.B., Bell, K.A., Phalen, R.F., Wilson, A.F., Ho, A., 1982. Growth-rate measurements and deposition modeling of hygroscopic aerosols in human tracheobronchial models. Ann. Occup. Hyg. 26, 93–108.
Mccallion, O.N.M., Taylor, K.M.G., Thomas, M., Taylor, A.J., 1995. Nebulization of fluids of different physicochemical properties with air-jet and ultrasonic nebulizers. Pharm. Res. 12, 1682–1688.
Miles, R.E.H., Knox, K.J., Reid, J.P., Laurain, A.M.C., Mitchem, L., 2010. Measurements of mass and heat transfer at a liquid water surface during condensation or evaporation of a subnanometer thickness layer of water. Phys. Rev. Lett. 105.
Mitsakou, C., Helmis, C., Housiadas, C., 2005. Eulerian modelling of lung deposition with sectional representation of aerosol dynamics. J. Aerosol Sci. 36, 75–94.
Morrow, P.E., 1986. Factors determining hygroscopic aerosol deposition in airways. Physiol. Rev. 66, 330–376.
Nerbrink, O.L., Pagels, J., Pieron, C.A., Dennis, J.H., 2003. Effect of humidity on constant output and breath enhanced nebulizer designs when tested in the EN 13544-1 EC standard. Aerosol Sci. Tech. 37, 282–292.
Prokop, R.M., Finlay, W.H., Stapleton, K.W., Zuberbuhler, P., 1995. The effect of ambient relative-humidity on regional dosages delivered by a jet nebulizer. J. Aerosol Med. 8, 363–372.
Reid, J.P., Dennis-Smither, B.J., Kwamena, N.O.A., Miles, R.E.H., Hanford, K.L., Homer, C.J., 2011. The morphology of aerosol particles consisting of hydrophobic and hydrophilic phases: hydrocarbons, alcohols and fatty acids as the hydrophobic component. Phys. Chem. Chem. Phys. 13, 15559–15572.
Reisner, C., Katial, R.K., Bartelson, B.B., Buchmeir, A., Rosenwasser, L.J., Nelson, H.S., 2001. Characterization of aerosol output from various nebulizer/compressor combinations. Ann. Allerg. Asthma. Im. 86, 566–574.
Watts, A.B., McConville, J.T., Williams, R.O., 2008. Current therapies and technological advances in aqueous aerosol drug delivery. Drug Dev. Ind. Pharm. 34, 913–922.
Xi, J.X., Si, X.H., Kim, J.W., Berlinski, A., 2011. Simulation of airflow and aerosol deposition in the nasal cavity of a 5-year-old child. J. Aerosol Sci. 42, 156–173.
Zhou, Y., Ahuja, A., Irvin, C.M., Kracko, D., McDonald, J.D., Cheng, Y.S., 2005. Evaluation of nebulizer performance under various humidity conditions. J. Aerosol Med. 18, 283–293.