The Mechanism of Breath Aerosol Formation

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Abstract:

Background: Aerosol production during normal breathing is often attributed to turbulence in the respiratory tract. That mechanism is not consistent with a high degree of asymmetry between aerosol production during inhalation and exhalation. The objective was to investigate production symmetry during breathing.

Methods: The aerosol size distribution in exhaled breath was examined for different breathing patterns including normal breathing, varied breath holding periods and contrasting inhalation and exhalation rates. The aerosol droplet size distribution measured in the exhaled breath was examined in real time using an aerodynamic particle sizer.

Results and Conclusions: The dependence of the particle concentration decay rate on diameter during breath holding was consistent with gravitational settling in the alveolar spaces. Also, deep exhalation resulted in a 4 to 6 fold increase in concentration and rapid inhalation produced a further 2 to 3 fold increase in concentration. In contrast rapid exhalation had little effect on the measured concentration. A positive correlation of the breath aerosol concentration with subject age was observed.

The results were consistent with the breath aerosol being produced through fluid film rupture in the respiratory bronchioles in the early stages of inhalation and the resulting aerosol being drawn into the alveoli and held before exhalation. The observed asymmetry of production in the breathing cycle with very little aerosol being

produced during exhalation, is inconsistent with the widely assumed turbulence induced aerosolization mechanism.

Keywords: Aerosol Distribution; bronchiole; Physiology; Breath Condensate; Exhaled Aerosol

Introduction:

According to the findings of the American Thoracic Society/European Respiratory Society Task Force (ATS/ERS TASK FORCE) on Exhaled Breath Condensate (EBC)[1], the important areas for future research include: ascertaining the mechanisms and site of exhaled breath condensate particle formation; determination of dilution markers; improving reproducibility; employment of EBC in longitudinal studies; and determining the utility of exhaled breath condensate measures for the management of individual patients. The first of these requirements concerns the identification of the source region and mechanism responsible for producing the aerosol present in exhaled breath. As stated by the authors of that report, the mechanisms which cause airway/alveolar fluid substances or those from the mucus layer to be added to exhaled breath are not clear and further study is required. These droplets of respiratory fluid may be released anywhere between the alveoli and mouth[2].

According to Wood eta al.[3]; *while there is an intuitive explanation for the presence of volatile substances in exhaled breath, the mechanisms by which non-volatile substances enter expired breath are poorly understood and need further investigation*.

Existing models of breath aerosol formation assume that turbulence, occurring in the respiratory tract results in the aerosolization of respiratory fluid [4-6]. That model does not adequately explain the influence that respiratory activities exert on aerosol concentration: Experimental data do not show a clear increase in aerosol concentration with exhalation flow rate during breathing, as should occur if turbulent airflow is the dominant breath aerosol formation mechanism. In fact normal breathing can produce higher aerosol number concentrations than coughing [4, 6, 7] which involves

far higher flow rates. Gebhart et al.[8] found that these particles are expired from volumetric lung depths of more than 200 cm^{-3} implying that they occur beyond the conducting airways. They also observed that the concentration decreased following breath holding, and suggested the opening of closed peripheral airways as a possible mechanism of generation of these particles.

Turbulence is associated with high Reynolds number (Re). During quiet breathing (<1 $L.s^{-1}$), airflow in the trachea is partly turbulent, with a Reynolds number of about 1500, however Re deceases deeper in the respiratory tract and flow in the bronchi is turbulent only at very high flow rates (5 $L.s^{-1}$) and it is laminar in the bronchioles at all naturally achieved flow rates[9]. Authors have previously pointed this out showing that aerosol production induced by turbulent flow is unlikely in the lower respiratory tract during normal breathing because airflow is laminar in the medium through to smaller bronchial airways down to and including the alveoli[10]. Therefore for normal breathing, the turbulence induced aerosolization mechanism must assume that aerosol formation occurs in the larynx or perhaps at bifurcations where local eddies can occur. Studies examining the composition of the aerosol question this assumption however.

Expired breath condensate contains non-volatile solutes produced primarily in the region of the deeper bronchiolar/alveolar region. Effros et al.[11] found that after adjusting expired breath condensate concentrations for dilution, and obtaining the original concentration of non-volatile solutes in the lining fluid (from which the aerosol was presumed to be derived), higher concentrations of Ca^{2+} cation were found than occurs naturally in plasma. This was attributed to the surfactant known to be generated in the distal parts of the lung, not the higher respiratory tract. Therefore this

material must be assumed to have been carried to the higher parts of the respiratory tract in order to retain the previous assumption.

Model description:

An alternate, very simple model of breath aerosol formation which addresses the above inconsistencies is described. The mechanism is based on a process of respiratory fluid film or bubble bursting during the clearance of fluid closures which form in the lower bronchioles following exhalation. The model is referred to as the bronchiole fluid film burst (BFFB) model.

The BFFB model relies on the following details concerning the physiology and physics of breathing which are well documented in the literature. A schematic representation of a section of bronchiole is shown in figure 1 where the processes discussed below are represented in a highly exaggerated way. During normal breathing in a healthy subject during exhalation, fluid which lines the surface of the elastic respiratory bronchioles, is expelled from spaces in the longitudinal folds in the bronchiolar surface as those spaces contract as per stages A-C in the figure. This process continues, eventually resulting in a more or less flat, continuous fluid layer over the interior surface of the bronchiole (B). It should be noted that in reality the respiratory fluid may contain air bubbles so that it resembles a foam rather than a pure liquid phase.

The surface tension of this now much reduced area of film, acts to draw the highly compliant airway closed even further. This is in accordance with the so-called Law of Laplace which shows that a small tube will experience a greater inward force (due to the surface tension of the lining fluid) than a large one even if their surface tensions

are equal. It must be noted at this point that a natural surfactant is present in this fluid and this acts to prevent the total collapse of most of the small airways because the surfactant, which reduces surface tension, is confined to the air water interface and therefore becomes more concentrated as the area of the interface decreases. Neverthesless, in some respiratory bronchioles, the remaining thin column of air collapses to such an extent that at some narrower points along the bronchiole the liquid film can completely block the airway (C). Due to the forces associated with fluid surface tension and reduced dimension, a higher pressure difference is required to reopen this airway, than was required to close it, even though this difference is substantially moderated by the presence of surfactant in the fluid[12]. The reopening of such closed airways occurs during the early stages of subsequent inhalation phase of the breathing cycle.

The following is a logical inference based on the above known physiology: During the reopening of a respiratory bronchiole, the fluid blockage will contract axially (transition from C to D in figure 1) as it is drawn radially outward by the expanding bronchiole, until the blockage is reduced to a thin membrane or bubble (state D in figure 1). The bubble subsequently bursts, reopening the airway (E). The film breakage will be accompanied by the fragmentation of fluid film as in the bursting of fluid bubbles generally. Film droplet aerosol production from bursting bubbles is well documented. For comparison seawater produces film droplets drying to equilibrium diameters in the range 0.25-2 μm[13]. These form at the ocean surface when bubbles of surfactant matter form and burst after entrained air bubbles rise to the ocean surface.

This above model is consistent with the observation that aerosol production increases as breathing becomes deeper and faster[6], since deeper exhalation is accompanied by greater reduction in bronchiolar diameter, and therefore a greater number of fluid blockages will be reopened.

Experimental evidence supporting the film droplet production mechanism as a source of aerosol droplets in exhaled air during normal breathing is presented in the following section.

Materials and Methods

Subjects

The experimental study was fully scrutinised and cleared by the Queensland University of Technology Human Research Ethics Committee. Subjects aged between 19 and 60 years of age were recruited via a broadcast email invitation offering a small financial reward. Subjects were instructed to self exclude if they were smokers, experiencing illness, asthma sufferers, had recently experienced expiratory problems or were likely to experience discomfort in confined spaces. In total 17 subjects participated in the study.

Study Design

Objectives

The primary study objectives were:

- To determine whether breath aerosol is produced equally during the inhalation and exhalation phases of breathing.

- To determine the influence of exhalation depth on the droplet number concentration of breath aerosol in the subsequent breath.
- To identify approaches for controlling and enhancing the production of breath aerosol through modified breathing patterns as a potential means to facilitate studies involving the collection and analysis of expired breath condensate and expired aerosol.

An additional objective was to detect any correlation of breath aerosol concentration with age.

Overview of experiments

Subjects were asked to perform several respiratory activities, during which the aerosol size distribution and concentration were measured. Water vapour concentration was used as a tracer of dilution during these measurements so that the concentration within the respiratory tract could be determined.

Methods

This research utilised the Expiratory Droplet Investigation System (EDIS) which is described in detail elsewhere[14]. The EDIS, shown schematically in figure 2, is a small wind tunnel 0.5 m in diameter, into which a subject can comfortably insert their head. HEPA filtered, recirculating air is propelled by a filter/fan module past the subject at a very low, controlled velocity. The particle free air carries any aerosol emitted by the subject to instrument sampling ports positioned inside the duct at a set distance downwind. The EDIS operates at slightly higher than ambient pressure to ensure that no ambient aerosol enters to contaminate the sample.

Aqueous aerosols dry rapidly to an equilibrium diameter determined by the relative humidity at the point and time of measurement and the hygroscopicity of the non-volatile components of the aerosol material. In the case of respiratory fluid these may include hygroscopic salts such as NaCl and organic materials including surfactant which may also affect the rate of evaporation. The aforementioned study by Morawska, et al.[14] showed that respiratory aerosols examined using the APS in the EDIS have achieved their equilibrium size when measured.

Aerosol size distributions in the diameter range 0.5-20μm were measured using an Aerodynamic Particle Sizer (APS) (TSI model 3312A). The APS measures the aerodynamic particle size distribution, which assumes unit density particles, spherical in shape. The aerodynamic diameter is likely to be a good approximation to the physical size both for droplets which are primarily aqueous and for dried residue particles, comprised mainly of organic matter if these have formed through the drying of initially spherical droplets and retain this shape on drying. Additional instrumentation included a relative humidity probe (Hygropalm Hycroclip) incorporated into the UV-APS probe tube extension to determine the water vapor concentration in the sample stream and a hot wire anemometer probe (TSI Velocicheck™ 8340) which determined the air velocity in the duct during aerosol measurements.

Breath emissions have been linked to depth in the respiratory tract by examining the time of emission within the breathing cycle. This approach requires a sufficient concentration of the target emission to overcome background noise in concentration reading. This was not possible with the APS aerosol concentration measurements

because of the very low concentrations involved, the small sample flow rate. The current approach instead uses only the average exhalation concentration per breath.

Aerosol dilution (D) was estimated via Equation 1 and

Equation 2 using water vapour concentration as a tracer and the resulting dilution factors were used to correct for dilution and obtain droplet number concentration in the respiratory tract.

Equation 1: Calculation of the sample dilution factor from water vapour concentration

$$D = \frac{AH_0}{AH_s - AH_{BG}}$$

where

AH_0 = water vapor concentration in the mouth during exhalation

AH_s = water vapor concentration in the sample

AH_{BG} = background air water vapor concentration in the EDIS

Equation 2: Calculation of absolute humidity or water vapour concentration from relative humidity and temperature

$$AH(T, RH) = \frac{RH}{100}\left(\frac{p_{sat} MW_{H_2O}}{RT}\right)$$

RH = relative humidity (%)

$p_{sat}(T)$ = saturation vapour pressure of water at T

MW_{H_2O} = molecular weight of water

R = gas constant

T = temperature (K)

The accuracy of the dilution correction depends on the accuracy of the assumed water vapour concentration inside the mouth during exhalation which we treat as a fixed value (3.3x10-2 kg.m-3), derived from published data[14-18]. Based on the wide

variations in the published data and in the accuracy of the water vapour concentration measurements, we ascribe an uncertainty to the dilution factors of 30 %.

Size distribution measurements for each subject, were conducted over three sessions at approximately 2 hour intervals. The sessions consisted of a sequence of activities defined in table 1, with each activity lasting 2 minutes. The sessions were repeated three times, with 20 minute rest periods between the sessions. The resulting size distributions were corrected for dilution to obtain the concentration in the respiratory tract. The average size distribution was then calculated for each activity across all subjects.

For normal breathing, the average breathing cycle duration based on breath count and activity duration, ranged from 1.7 to 10.9 s with an average of 4.1 s. This is not the same as the duration of inhalation and exhalation since that includes a brief (about 0.5 s) pause after exhalation. Allowing for this pause, the duration of the average normal inhalation/exhalation is approximately 3.5 s.

Inhalation via the mouth was employed during the modified breathing patterns b-3-0-f1–m-m, b-1-t-f3-m-m and b-3-0-f3–m-m (table 1) because it was necessary for rapid inhalation which would otherwise be restricted by the nasal passages in nasal inhalation.

Results

Depth and rate of exhalation

Figure 3 shows the average concentration as well as the average of the concentration enhancements over normal breathing for breath aerosol concentration during four

breathing patterns. All modified breathing patterns gave rise to a significant enhancement over normal breathing. The enhancement was attributed to the deep exhalation in those activities which did not occur during normal breathing. There was not a statistically significant difference between the enhancements obtained for rapid exhalation and slow exhalation. In contrast to this, rapid inhalation produced a significant and substantial increase, reaching 30 times the normal breathing concentration for one subject.

Deep exhalation prior to drawing a breath resulted in an increase in the average concentration across all subjects by a factor of 5.5 ± 3.5.

Fast inhalation versus fast exhalation

The role of flow rate was investigated by comparing aerosol production during fast inhalation with that during fast exhalation. Figure 4 shows the average size distribution obtained from the activity b-1-0-f3-m-m (rapid inhalation, no breath holding, full slow exhalation, table 1) and b-3-0-f1-m-m (slow inhalation, no breath holding, full rapid exhalation) for two subjects. The average size distribution for normal breathing (b-n-m) is shown for comparison. In both subjects deep exhalation produced clear increases in the concentration over normal breathing; however the increase was much greater with rapid inhalation than with rapid exhalation.

Gravitational deposition in the alveoli of aerosol generated through inhalation.

Figure 5 shows the average measured size distribution for young (age ≤ 35) subjects for breathing with full deep exhalation and subsequent rapid inhalation over a 1 second period, followed by breath holding for 0 s, 2 s, 5 s and 10 s, then exhalation over a 3 s period.

The size distribution shows distinctive erosion of particle concentration, which progressively moves from large to smaller sizes as the duration of breath holding increases. This pattern is strongly suggestive of gravitational settling where larger particles which fall with greater terminal velocity are lost earlier. This is potentially strong evidence that the aerosol is formed primarily during the inhalation rather than the exhalation phase of breathing.

Most of the available storage volume of the lung is in the alveolar spaces, and the dimension and geometry of these spaces is known to be a multifaceted prism which approximates a spear of diameter 0.2 mm. Gravitational settling in such a space can be modelled using equation 1 which describes the decay in particle concentration with time for a well mixed aerosol inside a spherical volume[19].

Equation 1

$$C(t) = C_0 \exp\left(\frac{-0.75 v_s t}{D}\right)$$

where

$C(t)$ = concentration at time t

C_0 = initial concentration

t = time

v_s = settling velocity

$$= \frac{\rho_p D^2 \times 10^{-12} \times 9.81 C_c}{(18 \times \vartheta)}$$

D = particle diameter (μm)

ρ_p = density of the particle

C_c = Cunningham slip correction factor

ϑ = vsciosity of air

Also shown in Figure 5 are the predicted size distributions based on the 0 s breath holding case, assuming gravitational settling in a well mixed 0.2 mm diameter spherical volume similar to that of the alveoli. Here we have adopted the hygroscopic growth assumption employed by Nicas et al.[20], who showed that droplets in the respiratory tract shrink by a factor of roughly 0.5 when exposed to ambient relative humidity. Therefore the diameter of the droplets in the saturated environment of the alveoli is assumed to be two times larger than the size measured. Figure 6 shows the result for a single subject using the same model assumptions.

The size distribution is affected by the detection efficiency of the 3320 APS which is known to decline from 100% at 0.9 μm to about 30% at 0.5 μm μm[21]. This accounts for the increasing discrepancy with decreasing diameter, between the modelled and measured values below 1 μm.

Correlation of Breath aerosol concentration with age of subject.

Figure 7 shows the relationship between breath aerosol concentration and subject age for normal breathing. A positive and statistically significant correlation ($p=0.02$) was observed between concentration and subject age, implying that breath aerosol production increases with age. It should be noted that most of the subject ages are located in a cluster at the lower end of the age range and this may reduce the validity of the statistical test. However it should also be noted that the eldest subject tested (identified as an outlier or "super emitter") produced concentrations more than an order of magnitude greater than the mean, and this subject was excluded from the above regression analysis on the assumption that their lung function was different in

some way from the remaining group. No conventional lung function testing was performed on the subjects.

Discussion

When interpreting the concentration data for contrasting depths and rates of exhalation it should be noted that the cartilaginous airways become somewhat compressed during forced expiration[22]. This will enhance the increases in velocity and turbulence expected during fast exhalation. It is therefore likely that a greater proportion of larger droplets are lost through airway inertial impaction during forced exhalation than during fast and normal exhalation. However in the airways, particle loss through inertial impaction occurs primarily for particles or droplets larger than 5 μm[23], and this occurs on surfaces in the upper respiratory tract where the velocity is higher. The vast majority of the droplets observed in this study were smaller than 2 μm so impaction losses in terms of the faction of total particle number lost are likely to be small. This is confirmed in figure 3 which shows that impaction losses did not significantly alter the number concentration when only the exhalation rate was changed, although a small statistically non-significant decrease in the concentration enhancement with respect to normal breathing can be seen for fast exhalation.

It is also important to note that deep exhalation is usually followed by deeper than normal inhalation, so although the inhalation rate was controlled to match as closely as possible, the duration of normal breathing inhalation, the volume of air inspired during a 3 second inhalation was probably greater following deep exhalations than in normal breathing. Therefore it is possible that the increased concentrations observed following deep exhalation were partly due to a higher flow rate during inhalation.

Nevertheless, the observation that rapid inhalation produced much greater increases in concentration than occurred with rapid exhalation is not consistent with a model employing turbulence induced aerosolization in the higher respiratory tract. Furthermore, in the higher respiratory tract the airways are supported by a more rigid cartilaginous reinforcement, and so are less pliable and do not close readily[12]. In fact as previously discussed, these airways may compress during forced expulsion, so it is probable that air velocity in this region is actually higher for fast exhalation than is the case for fast inhalation.

Given the longer residence time in the respiratory tract for aerosols generated during inhalation the turbulence mechanism should have produced the opposite outcome to that observed as greater concentrations would be expected for fast exhalation than for fast inhalation.

Interpretation of the results in terms of the aerosol formation mechanism.

The increased concentration observed for deep exhalation supports the proposed BFFB mechanism where the aerosol is formed from fluid blockages of the bronchioles since these should become more common as the lung becomes more deeply deflated.

The observation that there is no consistent increase in aerosol production when the exhalation rate is increased by a factor of three, supports the concept that the aerosol is not produced by turbulence in the airways, as this would produce aerosol equally during inhalation and exhalation.

The observation that aerosol production increases when the inhalation rate is increased by a factor of three supports the concept that the aerosol is produced during the inhalation phase of breathing. Rapid inhalation will produce a greater pressure drop across the bronchiole fluid closures so that a greater proportion of these closures are expected to reopen during fast inhalation. Therefore, the observation of increased production is also consistent with the fluid closure and film burst aspect of the model.

The observation that gravitational settling losses increase with increased breath holding duration and that this behaviour is consistent with the droplet size and the dimension of the alveoli, further supports a model where aerosol is produced mainly during the inhalation phase of breathing.

It is worth noting that Edwards et al.[7] showed that delivering an aerosolised surfactant solution to the lungs increases exhaled aerosol concentration during normal breathing 30 fold. Surfactants have a well known ability to increase aqueous film elasticity, thereby allowing bubbles to form readily and to achieve much larger diameters before bursting. This may also be consistent with the BFFB model, as bubbles will burst later in the inhalation phase when they, along with the bronchiolar diameter, are much larger.

When interpreted in the context of the BFFB model the existence of a positive correlation between breath aerosol concentration and age appears to suggest age related changes in the bronchiole structures, changes in the surfactant properties of the lining fluid or changes in breathing pattern which accentuate the BFFB process.

In summary, breath aerosol production occurs during the initial stages of inhalation, and the resulting aerosol concentration is proportional to the fraction of fully contracted bronchioles with blockages at the onset of inhalation and inversely related to the duration of breath holding.

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Disclosure Statement

No conflicts of interest exist.

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Table 1: Breathing activity labels and their definitions

Activity label	Activity Description
b-n-m	Breathing normally, inhaling via the nose and exhaling via the mouth, repeated for 2 minutes.
b-3-0-f1-m-m	Inhaling a normal* breath volume via the mouth over a 3 s period, followed immediately by a 1 second, full, deep exhalation via the mouth. Repeated for 2 minutes.
b-1-t-f3-m-m	Rapid inhalation of a normal* breath volume via the mouth over a 1 s period, followed by breath holding for a period of t s, then full, deep exhalation via the mouth over a 3 s period. Repeated for 2 minutes.
b-3-0-f3-m-m	Inhaling a normal* breath volume via the mouth over a 3 s period, followed immediately by a 3 second full, deep exhalation via the mouth over a 3 s period. Repeated for 2 minutes.

- *normal breath volume* means a normal breath volume as judged by the subject.

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Figure 1: Conceptual illustration of the BFFB mechanism occurring in a bronchiole

Figure 2: Conceptual schematic of the EDIS wind tunnel.

Figure 3: Mean concentrations and corresponding concentration enhancements over normal breathing, for three repeats with 13 subjects under the age of 36y, for four breathing patterns. The error bars show the standard error of the mean concentration.

Figure 4: Average dilution corrected expired aerosol size distribution for 2 subjects.

Figure 5: Average measured size distribution for young subjects for full exhalation with subsequent rapid inhalation over a 1 second period followed by breath holding for 0 s, 2 s, 5 s and 10 s, then exhalation over a 3 s period. Also shown are the predicted size distributions based on the 0 s breath holding case, assuming gravitational settling in a well mixed 0.2 mm diameter spherical volume (similar to that of the alveoli) assuming that the measured droplet diameter is half that of the droplets when in the saturated environment of the alveoli.

Figure 6: Measured size distribution for a 41 year old subject for rapid inhalation over a 1 second period followed by breath holding for 0 s, 3 s and 10 s, then full (deep) exhalation over a 3 s period. Also shown are the predicted size distributions based on the 0 s breath holding case, assuming gravitational settling in a well mixed 0.2 mm diameter spherical volume (similar to that of the alveoli) assuming that the measured droplet diameter is half that of the droplets when in the saturated environment of the alveoli.

Figure 7: Average breath aerosol droplet number concentration versus subject age.