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HIGH FREQUENCY ULTRASONIC ATOMIZATION FOR DRUG DELIVERY TO RODENT ANIMAL MODELS. OPTIMAL PARTICLE SIZE FOR LUNG INHALATION OF DIFLUOROMETHYL ORNITHINE

Guifang Zhang¹, Chris Fandrey², Amir Naqwi², and Timothy Scott Wiedmann^{1,3}

¹Department of Pharmaceutics, University of Minnesota, Minneapolis, MN 55455

²Powerscope Incorporated, 1313 Fifth Street SE, Suite 209, Minneapolis, MN 55414

Abstract

A high (8 MHz) and low (1.7 MHz) frequency ultrasonic transducer were compared for delivering aerosols to mouse lung. The aerosol concentration (mass of dry particles/volume of air) rose nonlinearly with solution concentration of difluoromethyl ornithine for both transducers. The particle size was linear with the cube root of the solution concentration, and the slope of the low frequency transducer was eight times greater than that of the high frequency transducer. The deposition fraction assessed by the assayed mass in the lung relative to the calculated inhaled mass was found to decline exponentially with particle size. The lower frequency transducer provided a higher dose despite a lower deposition fraction, but the high frequency transducer was more efficient and provides a more selective deposition in the lower respiratory tract while operating with significantly less demands on aerosol drying.

Keywords

Ultrasonic atomization; DFMO; Particle size distribution; Output; chemoprevention; aerosol delivery

INTRODUCTION

Aerosol inhalation is a direct method of drug administration for treatment of respiratory diseases (1,2). The great advantage lies in the fact that the drug deposited from inhalation results in a high concentration at the site of action. In addition, a much lower concentration is produced in the systemic circulation following absorption. This advantage in enhanced local concentration and reduced systemic concentration will scale approximately with the lung weight relative to the whole body weight. Since, the lung constitutes a rather small percentage of the total body weight, aerosol administration represents a significant advantage in terms of delivery efficiency even with a relatively low deposition fraction.

³To whom correspondence should be addressed: Timothy Scott Wiedmann, Ph.D, University of Minnesota, Department of Pharmaceutics, 308 Harvard St. SE, Minneapolis, MN 55455, Phone 612-624-5457, Fax 612-626-2125, wiedm001@tc.umn.edu.

The full potential of inhalation drug delivery is presently difficult to ascertain. Rodent animal models are preferred for early safety and efficacy studies; however, delivery systems developed for use in humans are not very effective for delivering aerosols to rodent animal models. The reason is that rodents, including mice, rats, and hamsters, are obligate nose breathers and as such, aerosol particles should be less than 1 μm to avoid overwhelming deposition in the nasopharyngeal area (3). In contrast, maximal deposition in humans occurs when the aerosol particle size falls in the 1–5 μm size range (4). Thus, the use of human delivery systems in rodents will result in perhaps as much as 99 % of the drug depositing in the nasopharyngeal region of the respiratory tract and only 1 % in the lung. This will lead to a gross underestimation of the effectiveness of the drug in animal preclinical efficacy testing from a simple view of response relative to an inhaled dose of drug. Even worse, the drug deposited in the nasopharyngeal region of the respiratory tract can lead to pronounced systemic side effects, which leads to an overestimation of the toxicity of the drug given by inhalation. Thus, there is an immediate need for an aerosol delivery system that can generate small particles (submicron) that would maximize the deposition of the drug in the lower respiratory tract of rodents.

With this in mind, we have examined the use of ultrasonic atomizers as a means to generate small aerosol particles that would be suitable for delivery to rodents. Although the physics of the actual aerosol production remains challenging to describe theoretically, there is a considerable amount of data that can be used to identify suitable ultrasonic transducers (5–11). That is, the particle size and output for specific ultrasonic devices have been empirically related to the physical properties of solutions (5,10,11). For solution properties, the viscosity, surface tension, density, and vapor pressure have been correlated with the output and particle size (5). In addition, the power and frequency are the primary factors of the ultrasonic transducer in determining the properties of the aerosol cloud.

In this study, we have used a high frequency (8 MHz) ultrasonic transducer to generate aerosol particles in the submicron size range of difluoromethyl ornithine (DFMO), which is a candidate for chemoprevention of lung cancer, and have determined the output, particle size and deposition in a mouse animal model (12,13). For comparison, we have made analogous measurements of the same parameters with a 1.7 MHz transducer. A third transducer operating at 2.35 MHz was characterized but not used in animal experiments. The results are generally in accord with previous theoretical predictions. More importantly, we have identified the operational constraints of varying the frequency of ultrasonic atomizers for evaluating the safety and efficacy of drug delivery by inhalation.

EXPERIMENTAL

Materials

Difluoromethyl ornithine (DFMO) was provided by NCI. Dansyl chloride (DNS) and glacial acetic acid (Analytical Reagent) were purchased from Mallinckrodt. Sodium acetate anhydrous was purchased from Fisher. All organic solvents were HPLC grade, and the water was deionized and distilled in an all-glass apparatus.

Methods

Physical properties—The viscosity was determined with a Cannon-Fenske calibrated capillary viscometer (Cannon Instrument Company, State College, PA). The specific gravity was measured with a 10 ml pycnometer (Fisher Scientific) using deionized, distilled water as reference. The density of DFMO powder was also determined with the pycnometer based on the displacement of heavy mineral oil, which readily wets but is a poor solvent for DFMO. The surface tension was determined using a platinum du Noüy ring and manual tensiometer (Central Scientific Co., Chicago, IL) and the results are expressed as a ratio of the observed surface tension to that of pure water. The vapor pressure was determined with a HygroLab 3 (Rotronic Instrument Co., Huntington, NY). The observed vapor pressures were converted to the relative water activity using the literature value of the vapor pressure at the measured temperature. All measurements were carried out at room temperature $22 \pm 0.5^\circ\text{C}$ and in triplicate.

Ultrasonic transducers—A 1.7, 2.35, or 8 MHz transducer was used to generate the initial droplets of a DFMO solution. The 1.7 MHz transducer was purchased from Mainland Mart, mainlandmart.com. The 8 MHz transducer was custom-built with support from Boston Piezo Optics, Boston MA and Department of Electrical Engineering and Computer Science, University of Minnesota (Professor Emad Ebini and Dr Hanwoo Lee). The 8 MHz transducer was driven with a WaveTek frequency generator (BK Precision 4040A, B&K Precision Corporation, Yorba Linda, CA 92887 U.S.A.), and the signal was amplified with an EIN RF power amplifier (model 350L). A transducer with a 2.35 MHz frequency was also built in order to obtain data at an intermediate frequency.

The relationship between electrical power input and the acoustic power output of the high frequency transducers was measured by an Ultrasound Power Meter (Ultrapower meter, model UPM-DT-1400AV, Ohmia Instrument Co., Easton, MD 21601). This device uses a force balance to measure the acoustical energy produced by a transducer submerged in a water bath. For the 8 MHz transducer, the output power was linearly related to the input electrical power with an efficiency of 19 %. The 1.7 MHz transducer had a fixed output of 10.3 W with a labeled electrical input of 28.8 W.

Aerosol generation and characterization—For particle generation, a Pyrex glass baffle was constructed (University of Minnesota, Department of Chemistry Glass Shop) and placed in the water bath, directly over the ultrasonic transducer. Air was directed into the baffle and adjusted to a flow rate of 0.5 L/min as measured by an inline flow meter. The air entrained the aerosol droplets containing a drug solution and carried the particles into the drying column. A 1.7 MHz transducer (Mainland Mart, mainlandmart.com) was used to generate the initial droplets of a DFMO solution, which were subsequently dried by either a reflux drying column (13,14) or silica drying column (15). Aerosols generated with the higher frequency transducers were dried with a silica drying column (TSI, Minneapolis, MN).

The concentration of DFMO in the baffle (referred subsequently as the solution concentration, $[\text{DFMO}]_{\text{soln}}$), generation process, and duration of exposure were varied to

achieve the desired particle size distribution and mass deposition for analytical measurement of DFMO in the lung. For the 1.7 MHz transducer, the mass output rates were measured by collecting particles for fixed periods of time with microfibrile filters held in plastic cartridges at the exit point of the drying column. The output was generally determined gravimetrically by weighing the filters immediately after collection as well as after drying in an oven. The mass of DFMO was also measured by HPLC to verify the chemical stability of DFMO. There was no significant difference among these methods, and thus the mass output rate was assumed to be solely due to dry DFMO particles.

In reporting the results, the aerosol concentration, $[\text{DFMO}]_{\text{aerosol}}$, refers to the mass of DFMO per unit volume of air and was obtained by dividing the mass output per unit time by the air flow rate

$$[\text{DFMO}]_{\text{aerosol}} = \text{Mass output rate} / \text{air flow rate}$$

Another parameter that was calculated to facilitate the comparison of the outputs of the different atomizers is the liquid output rate, Q_1 . This was calculated as the mass output rate of DFMO divided the baffle solution concentration,

$$Q_1 = \text{Mass output rate} / [\text{DFMO}]_{\text{soln}}$$

This represents the rate of production of liquid droplets assuming that the aerosol droplets that were initially produced have the same concentration of DFMO as the baffle solution.

For the 1.7 MHz transducer, the particle size distributions were determined with an Anderson Mark II cascade impactor. Collections were made at the point just prior to the entrance of the animal chamber, and the mass collected was determined gravimetrically. The mass median aerodynamic diameter (MMAD) and associated geometric standard deviation (GSD) was calculated from linear regression of an X-Y probability plot of the cumulative undersized mass as a function of the logarithm of the cutoff diameter using Kaleida-Graph software. The values were based on the pooling of three independent measurements.

For the 8 MHz transducer, the output and particle size distribution of the dry particles were determined with a scanning mobility particle analyzer (SMPS, model 3034, TSI, Minneapolis, MN) after isokinetic dilution of the aerosol particles with air. The aerosol concentration was determined as the product of the particle volume using the volume mean diameter and assuming a spherical shape and the number density of the particles expressed as count per liter of air after correction for dilution.

Inhalation experiments—For the one minute exposures, the rodents were hand held such that only their nose was within the aerosol stream for the duration of the exposure. For the 8 and 10 min exposures, a four-port or eight-port chamber with cylindrical animal restraining tubes was used to provide a “nose only” exposure. For the 15 minute exposure using 8Hz transducer, a single port chamber was used. The animals used in aerosol exposure were

female A/J mice (21–27 g) purchased from Jackson Laboratories (Bar Harbor, Maine). They were fasted overnight but allowed water ad libitum. Following exposure, the animals were sacrificed by CO₂ suffocation. Blood was obtained by cardiac puncture and was collected into plastic centrifuged tubes. After coagulation, the tubes were centrifuged and the serum removed and stored at –20 °C until assayed. The lung was severed at the carina, the esophagus and trachea were removed, and all stored at –20 °C until assayed.

HPLC analysis of DFMO in tissues—DFMO in lung tissues and serum was quantified by HPLC following derivatization with dansyl chloride by a modification of the procedure of Cohen et al (16). DMFO was assayed in lung by first weighing each lung. The lung was then homogenized following the addition of 500 µl PBS solution. A 250 µl aliquot of the homogenate was added to 20 µl of the 100 µg/ml 4-amino-2-hydroxy butyric acid as the internal standard solution. Then, 1000 µl methanol and 500 µl acetonitrile were added, the sample was mix thoroughly and centrifuged at 13,000 rpm, 4 °C for 15 minutes. The supernatant was removed and dried under nitrogen. The dried sample was reconstituted with 200 µl NaHCO₃-NaCO₃ 0.5 M buffer solution (pH = 8.5) and 200 µl of a 6 mg/ml dansyl chloride in acetone was added. After mixing, the solution was kept in dark for at least 12 hours, and the reaction was stopped by adding 200 µl water adjusted to a pH of 8.5 with sodium hydroxide, and 500 µl CH₂Cl₂ was added to extract the excess dansyl chloride by vortexing for 1 minute. After centrifuging at 13,000 rpm (4 °C) for 10 minutes, a 20 µl aliquot of the supernatant was injected onto the HPLC. The mobile phase consisted of methanol, acetonitrile, and a sodium acetate solution (10 mM) adjusted to a pH of 3.8 by the addition of acetic acid glacial in a volume ratio of 21:17:63. The flow rate was 1.2 ml/min, and the detection wavelength was 330 nm. The HPLC system consisted of a Shimadzu LC-10AT solvent delivery module, SIL-10AD auto injector, SPD-6AV UV-Vis spectrophotometric detector, and C-R5A Chromatopac integrator (Shimadzu Corp., Kyoto, Japan). An octadecyl silane (ODS) SUPELCO (250 mm × 4.6 mm, 5 µm) column was used in all separation experiments. The extraction efficiency of DFMO was 98 % for the lung.

Pharmacokinetic analysis—Calculations were carried out with MS Excel spreadsheet. The zero order delivery rate (mass/unit time) of inhaled DFMO aerosol, k_{inh} , was calculated as follows (13):

$$k_{inh} = [DFMO]_{aerosol} * RMV$$

where $[DFMO]_{aerosol}$ is the aerosol concentration of DFMO given in units of dry mass/liter of air, and RMV is the respiratory minute volume of the mouse taken to be 0.025 l/min based on Guyton's formula (17). The aerosol concentration was calculated from the filter collections above or SMPS data, combined with the measured air flow rate.

The mass deposited was corrected for the lung clearance using the reported half-life of lung clearance of 35 min (13). The lung concentration is assumed to rise in a first order manner secondary to a zero order deposition of DFMO characterized by the rate constant, k_{dep} . The following equation provides the deposition rate corrected for lung clearance:

$$k_{\text{dep}} = KM_{\text{lung}}[\text{DFMO}]_{\text{lung}} / \{1 - \exp(-Kt)\}$$

where $[\text{DFMO}]_{\text{lung}}$ is the weight/weight concentration of DFMO in the lung at a time, t , which represents the exposure time. M_{lung} is the lung mass. The deposition fraction, F , was calculated as the mass of drug deposited in the lung relative to the inhaled mass after correcting the lung levels for the clearance of drug that took place during the exposure. That is,

$$F = k_{\text{dep}} / k_{\text{inh}}$$

RESULTS

Figure 1 provides the results obtained for the physical properties relevant to atomization of the DFMO solution. DFMO is a low molecular weight compound with a structure similar to lysine. As such, the minor changes in the density, vapor pressure, and surface tension observed with increasing DFMO concentration are consistent with expectations. The viscosity was the property most greatly affected by the concentration, but even here, the value was less than a factor of two greater than water at a concentration of 300 mg/ml.

In Figure 2a, the aerosol concentration of DFMO is given as a function of the solution concentration for the 1.7 MHz ultrasonic transducer. The aerosol concentration was determined from the mass output per unit time divided by the air flow rate. As the solution concentration is increased, the mass of DFMO in the aerosol droplets also increased. While the aerosol concentration increases with solution concentration, the effect is evidently non-linear. Thus, it appears that the rate of production of droplets decreases as the concentration of DFMO increases possibly due to the increase in the solution viscosity. There may also have been a higher fraction of particles deposited in the tubing leading to capturing filters or possibly changes in the initial particle size of the droplet. This latter effect is addressed below.

The companion data obtained with the transducers at 2.35 and 8 MHz are shown in Figure 2b along with the low concentration data obtained with the 1.7 MHz transducer for comparison purposes. In a similar manner, the aerosol concentration increased as the solution concentration was increased. Due to limitations with the SMPS in collecting larger particles (i.e. particles >500 nm are removed before analysis), the solution concentration was limited to 10 mg/ml and lower. The aerosol concentration was much lower with the 8 MHz transducer than that observed with the 1.7 MHz and 2.35 MHz transducers. The curves of aerosol concentration as a function of solution concentration were fit to a second order polynomial with the intercept taken as zero, and the coefficients of the linear term were 0.053, 0.14 and 0.42 ml/L in order of decreasing frequency. These coefficients represent the total volume of aerosol droplets per volume of carrier gas, that is, the liquid droplet aerosol concentration. Thus, the liquid droplet aerosol concentration is seen to be about eight times larger for the 1.7 MHz in comparison to the 8 MHz transducer.

In Figure 3, the median aerosol size of the dried particles is given as a function of the cube root of the solution concentration for the 1.7 and 8 MHz transducers. For the lower frequency transducer, the particle size was obtained with a cascade impactor and is given as the mass median aerodynamic diameter (MMAD), whereas the particle size for the high frequency transducer was obtained with the SMPS and is given as the volume median diameter. In both cases, a straight line was obtained indicating that the dry particle size scales appropriately with the solution concentration. This observation is consistent with the initial droplet size being independent of the solution concentration. As such, the nonlinear increase in output rate observed with an increase in solution concentration is more likely related to a decrease in droplet formation rate secondary to an increase in viscosity, as suggested above, rather than a change in particle size or a change in the throughput efficiency of the drying column. However, the insensitivity of the cube root dependence of the particle size on output droplet size makes it difficult to unequivocally establish that there was no change in particle size with a change in solution viscosity.

In Figure 4, the logarithm of the deposition fraction in the lower respiratory tract of mice is given as a function of particle size. The line represents a best fit to an exponential function, which was chosen for the mathematical convenience in the analysis. Nevertheless, as the particle size was decreased, there was nearly an exponential increase in the deposition fraction rising from less than 1 % to nearly 50 % at a particle size of 150 nm.

DISCUSSION

In this study, the aerosol characteristics of two ultrasonic atomizers were compared for their use in inhalational delivery of drug to rodent animal models. The third atomizer (2.35 MHz) was also characterized, but animal experiments using this device were deemed unnecessary. For testing potential therapeutic modalities, the primary concern is the total mass of drug deposited in the lower respiratory tract. This is dependent on both the aerosol concentration of drug and the particle size, which in turn determines the fraction of drug deposited in the lung relative to the total dose.

For ultrasonic drivers, particles are ejected from the surface of the liquid and are entrained by the carrier gas. This process of ultrasonic generation has been described by two main theories, and a third that is a hybrid of the two (6,8,9). The capillary wave theory is based on the idea that droplets are ejected from the crests of the capillary waves when the sound wave exceeds a critical amplitude (9). The critical amplitude is a function of the sound wave but also the solution properties including the surface tension, density and viscosity.

The second, cavitation theory, describes atomization as being secondary to the formation and collapse of cavitation voids just beneath the liquid surface (7). The combination theory suggests that collapsing cavitation voids causes the ejection of droplets from surface capillary waves. The combination theory appears to accommodate the empirical correlations between the solvent properties and output as well as the dependence on particle size and frequency (5). To date, there are several rigorous models that have examined particle size. However, few have attempted to address the question of output. In fact, the above ideas capture the main aspects of the ultrasonic atomization process, but presently there are no

expressions in the literature that would allow quantitative prediction of the output from high frequency ultrasonic atomizers.

The present experiments can not allow a pure cavitation model to be distinguished from a combined theory. In the mid 1960's a group of Russian scientists did extensive work on the topic and concluded that cavitation was indispensable to the atomization process. In that study, the atomization rate was empirically correlated to the vapor pressure, surface tension, and viscosity (7,8). In analyzing the DFMO solutions, the only solution property that significantly changed was the viscosity. Indeed, the nonlinear increase in atomization rate as reflected in the plots of aerosol concentration as a function of solution concentration may be a result of the nonlinear increase in viscosity. Thus, a plot of the aerosol concentration as a function of the inverse square root of the viscosity yields a straight line, but the limited range of the viscosity in this study fails to provide a convincing case for ascribing a mechanism for the aerosol production.

From the standpoint of animal exposure studies, it would be useful to have an *a priori* value of the output rate, which may be related to the aerosol concentration and aerosol particle size, and thereby dose, based on the properties of the device and the solution. In principle, predicting the number density of droplets coupled with the droplet particle size provides a direct means to determine the aerosol concentration. However, the droplet ejection rate is unknown. A theoretical description of the ejection rate would require a quantitative description of the cavitation process that can be hydrodynamically connected to the surface disturbances, which would need to account to acoustic streaming and the formation of the fountain. In this study, the particle ejection rate does not appear to be constant with a changing frequency. There are also complications arising from the inherent experimental difficulty in achieving quantitative entrainment of all ejected droplets by the air flow as well as the likely variation in the area (i.e. fountain size) from which droplets are ejected. Finally, it should also be noted that concentrations above 10^7 particles per milliliter will undergo time dependent aggregation secondary to diffusional collision in a time scale relevant for inhalation experiments, which causes a change in the particle size distribution and represents an upper boundary for the rate of drug delivery to the respiratory tract.

To clarify the relationship of the deposited dose to the magnitude of droplet ejection rate and particle size, we note the following. For the 1.7 MHz transducer, a cascade impactor was used to measure the particle size. The particle size may be related to the initial droplet size, density and cube root of the solution concentration as follows, beginning with the assumption of mass balance of DFMO in the initial droplet, $M_{d,DFMO}$, and final dry particle, $M_{p,DFMO}$,

$$M_{d,DFMO} = M_{p,DFMO} \\ C(\pi D_i^3/6) = \rho_d(\pi D_p^3/6)$$

where ρ_d is the density of the dry particle, D_i is the volume median diameter of the initial drops, and C is the initial solution concentration in g/ml. Using the definition of the MMAD as being proportional to the geometric diameter and the square root of the particle density

$$\text{MMAD} = \rho_o^{1/2} \rho_d^{-5/6} D_i C_w^{1/3}$$

where ρ_o is the unit density. For the 1.7 MHz transducer, the slope was equal to $4.56 \times 10^{-4}[(\text{ml})^2/\text{g}]^{1/3}$ (Figure 4). The density of DFMO as a powder was measured to be 1.49 g/ml. Thus, the initial droplet size is estimated to be about 6 μm . Thus, it appears that there is aggregation of the initial droplets leading to the large initial size, since independent measurements of the initial droplet size were near 2.8 μm .

For the high frequency transducer, the SMPS was used to determine the particle size and number density. In this case, the observed measure of aerosol size is the volume mean diameter, D_v . The corresponding relationship between particle size and solution concentration is as follows:

$$D_v = \rho_d^{-1/3} D_i C_w^{1/3}$$

The value of the slope for the plot of the diameter as a function of the cube root of the solution concentration was 0.987. The estimated initial droplet diameter in this case is about 1.1 microns assuming a spherical shape. The analogous plot for CsCl, which has a density near 4 g/ml, had a slope of 0.93 (data not shown). The initial diameter is estimated to be 1.5 microns, which is similar to that found with DFMO as expected.

In comparing the 1.7 and 8 MHz, the initial particle sizes may be taken as being 2.8 and 1.1 microns. If the particle ejection rates are comparable, then the aerosol concentration for the 1.7 MHz transducer should be $(2.8/1.1)^3 = 16.5$ times greater, since the mass output varies with the cube of the particle size at constant solution concentration. However, the aerosol concentrations for these two transducers only differed by a factor of eight. Thus, the 8 MHz transducer produced an aerosol concentration two times higher than expected based on the 1.7 MHz transducer. Because of the inherent difficulties, the cause of the discrepancy can not be unequivocally determined. Nevertheless, the implication is that the high frequency device will be more efficient in generating aerosols for delivery of drug to rodent animal models.

For practical considerations in determining the safety and efficacy in animal studies, the dose is typically varied over a relatively large range to identify concentrations corresponding to the minimum and maximum effectiveness from which the effective dose for 50 % of the animals (ED50) can be found. Ideally, the mass of drug deposited in the lung should be varied with the aerosol concentration while all other parameters are kept constant. As such, the maximum dose should be produced at a specific exposure time and then lower doses can be delivered by diluting the aerosol concentration, which will allow the particle size, and with it the percent deposition and exposure time to be held constant.

However, the maximum dose is only a function of particle size given the upper limit in particle number density that can be achieved without significant aggregation. As seen in this study, there are opposing effects of deposition fraction and aerosol output on the deposited

dose. In figure 4, the relatively well known relation that deposition fraction rises with a reduction in particle size is corroborated in this study. However, as shown in Figure 3, the aerosol concentration is greater for devices that generate larger particles. Thus, there exists an optimal particle size that maximizes the dose to the animal at a fixed exposure time.

To find the maximal possible dose, the dose in unit time can be expressed as the product of the deposition fraction and aerosol concentration,

$$\text{Dose} = F * [\text{Drug}]_{\text{aerosol}}$$

In general, both the aerosol concentration and deposition fraction can be written as a function of the particle size, which for the latter in this study is, $\ln(F) = aD_v - b$, thus

$$\text{Dose} = [\exp(aD_v - b)] [\rho_d N_d \pi D_v^3 / 6]$$

where N_d is the number density of the aerosol cloud, and a and b are constants of the logarithm of the deposition as a function of particle size fit. The particle size that provides the maximum dose can be obtained by setting the derivative to zero and solving for the particle size, or

$$d(\text{Dose})/d(D_v)|_{=0} = [\exp(aD_v - b)] [\rho_d N_d \pi D_v^2 / 2] + [\rho_d N_d \pi D_v^3 / 6] [a \exp(aD_v - b)]$$

Solving,

$$D_v = -3/a$$

Thus, for the deposition data, the value of the slope is -0.64 . Thus, assuming a fixed number density, the optimal dry particle size is 4.7 microns.

However, with the present devices, the aerosol concentration was found to be a nonlinear function of the solution concentration, which in turn implies that the number density is not independent of particle size. Nevertheless, the dose as a function of solution concentration can be calculated from the fitted curves. That is, the aerosol concentration can be expressed as a second order polynomial with respect to solution concentration and the deposition fraction can be expressed as a logarithmic function of the particle size which in turn is given as a cube root function of solution concentration. Thus, the deposited dose is given as the product of these two fitted functions and this dose is plotted as a function of solution concentration in Figure 5 for the two transducers.

As can be seen, the low frequency transducer delivered a higher deposited dose than the high frequency transducer in the accessible concentration range. Although the high frequency device provided a much higher deposition fraction (by a factor of 5), the smaller particle size

resulted in a lower aerosol concentration (a factor of 10). As such, the low frequency device delivered about three times the dose at the peak at concentration of 10 mg/ml. Higher concentrations were not explored, but the falling output rate with increasing solution concentration indicates that even with a higher solution concentration, the high frequency device would not provide a much higher dose. From this analysis, it appears that the lower frequency transducer provides a higher dose for a given solution concentration.

Despite delivering a lower dose, the high frequency device dose offers a number of significant advantages. First, the deposition fraction with a concentration of 10 mg/ml was 0.3 for the high frequency device, whereas with the lower frequency device it was only 0.05. From Raabe et al (3), it can be expected that much of the remaining dose at the high frequency was exhaled. In contrast, the low frequency transducer would produce a particle size distribution such that much of the inhaled dose would be deposited in the nasopharyngeal region of the rodent. This represents a serious confounding factor for interpreting the safety and efficacy of aerosol delivery as well as extrapolation to human studies. A second consideration is the volume of solvent that must be evaporated. Since the particle size is 2.25 times larger with the 1.7 MHz transducer, over 12 times more solvent must be evaporated. This puts an additional demand on the purity of the water used in the solution. More importantly, sufficient capacity for long term drying must be available for exposing of larger batches of animals.

In summary, high frequency ultrasonic atomizers are capable of producing smaller aerosol particles that have a much higher deposition fraction in rodent animal models. The loss in output is largely compensated by a more selective deposition in the lower respiratory tract thus minimize the potential systemic toxicity. Because the deposited dose depends on both the particle size and aerosol concentration, which in turn is determined by the solution concentration and transducer performance, there is an optimal concentration for maximizing the delivery of drug to the lower respiratory tract of the mouse for a given transducer..

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Figure 1. Viscosity in cPs (◆), relative surface tension (▲), specific gravity (■), and water activity (relative vapor pressure) (x) as a function of DFMO concentration (mean $\pm$ SD, n=3).

Figure 2. DFMO output as a function of solution concentration (1.7 MHz transducer; Lower plot: three transducers with 1.7, 2.35 and 8 MHz frequencies).

Figure 3. Mean particle size as a function of the cube root of solution concentration (MMAD for 1.7 MHz transducer and volume mean diameter for 8 MHz transducer).

Figure 4. Deposition fraction of DFMO in mouse lung as a function of the median particle size. The line represents a best fit of the data assuming a logarithmic linear relationship.

Figure 5. Deposited dose of DFMO as a function of the logarithm of the solution concentration using the fitted output as a function of solution concentration.