Nebulization of NanoCrystals™: Production of a Respirable Solid-in-Liquid-in-Air Colloidal Dispersion

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Received October 21, 1996; accepted November 1, 1996
KEY WORDS: nebulization; NanoCrystals™; aerosol; respiratory drug delivery.

INTRODUCTION

Delivery of drugs to the respiratory tract is important for both local and systemic treatment of disease (1). With formulations intended to be delivered as aerosols, the efficiency of delivery of drug to the respiratory tract is largely determined by the particle size distribution of the aerosol droplets (2–4). If the formulation is a solid-in-liquid dispersion, the size distribution of the aerosol is, in turn, intricately related to the properties of the dispersion medium and the size distribution of the dispersed phase. It is evident that with atomization of such formulations, the solid particles must be incorporated into a respirable aerosol droplet in order to reach the intended site of delivery.

While stabilization of coarse suspensions can be challenging, stabilization of aerosol formulations of a solid-in-liquid dispersion for respiratory delivery is a far greater problem. The difficulty arises from the stringent requirements of respiratory deposition. Specifically, the aerosols produced should have aerodynamic diameters in the range of 1–5 μm (2–4). With larger aerosol droplets (>5–10 μm), deposition occurs primarily on the back of the throat which can lead to systemic side effects (5–9). Therefore, the solid phase particles must be stabilized at a size well below the respirable size of 1–5 μm so that the solid particles can be readily incorporated into the respirable aerosol droplets. Failure to provide a stable particle size distribution of the solid in the formulation will lead to variability in the aerosol droplet size and thereby variability in the respiratory deposition, which may have an undesirable therapeutic outcome.

In present dispersed systems, aerosol particles are not ideal, because the suspended drug typically has a mean particle size that is comparable to the aerosol droplet size. Thus, there is an unfavorable statistical probability of placing solid particles within respirable liquid aerosol droplets (10,11). To produce an ideal respirable aqueous suspension, solid particles in the liquid dispersion should be stabilized with a size distribution well under 1–5 μm size range. That is, suspensions must be stabilized to prevent the aggregation and particle growth which result from the large surface energies of particles with high curvature. Recently, solid particles with a size in the nanometer range have been achieved through the use of NanoCrystal™ technology, but the application of this technology to respiratory drug delivery has not been explored (12). Therefore, the nebulization of NanoCrystals™, a solid-in-liquid aqueous dispersion stabilized as a small particle size distribution, has been studied for its use in respiratory drug delivery.

EXPERIMENTAL

Materials

Beclomethasone dipropionate (BDP) and polyvinyl alcohol (PVA), molecular weight 30,000–70,000, were obtained from Sigma Chemical Co. (St. Louis, MO) and used as received. All other chemicals were analytical/reagent grade or better.

NanoCrystal™ Dispersions Preparation and Characterization

The NanoCrystal™ dispersions were prepared by ball milling a suspension of 5% (w/v) beclomethasone dipropionate in an aqueous solution of 2.5% (w/v) PVA until no further decrease in particle size was achieved (24 hrs) (12). The intensity averaged particle size distribution was determined by a N4MD coulter laser light scattering analyzer. The size intensity distribution was periodically monitored throughout the course of the study. The particle sizes were also determined for each dilution.

The solubility of micronized BDP was determined in water by placing excess micronized BDP (1–5 mg) into Teflon™-lined screw capped glass test tubes along with 2–3 ml of 0.1% (w/v) PVA or 2.5 (w/v)% PVA. The tubes were periodically shaken while they were allowed to equilibrate at room temperature for three days. The suspensions were centrifuged, and the absorbance was determined at 240 nm by a Beckmann DU 74 spectrophotometer.

The specific gravities of 0.1, 0.5, 1, and 2.5% PVA were estimated with a 3 ml volumetric flask. The volume was calibrated with double distilled water, and the weight was then determined. The relative viscosity of these solutions was also determined with a capillary viscometer (1EA #7162 N10, A.H. Thomas, Philadelphia, PA) at 23.7 ± 0.2°C. The time for flow was measured in triplicate.

Nebulization

Prior to nebulization, the air flow through the nebulizer was determined as a function of pressure. A pressure gauge was placed in line between the pressure regulator and nebulizer. The air flow rate was measured down line from the nebulizer with a calibrated flow meter (Dwyer, Michigan City, IN). All nebulization experiments were conducted at an air flow rate of 6 lpm and an operating pressure of 15 psig for the nebulizer.

The set up for evaluating the aerosol production process consisted of a gas cylinder of compressed air as the source, which was equipped with a pressure regulator (Fisher Scientific Co.). Oxygen connecting tubing joined the regulator to the

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0724-8741/97/0100-0112$12.50/0

Salter Labs #8900 nebulizer (Arvin, CA, received as a gift from the manufacturer). A T-connector was placed on top of the nebulizer, and one of the exit ports was blocked with a #2 rubber stopper. During the nebulization experiments, the second exit port was fitted with a second T-connector which had an 18 mm diameter. The lower opening of the T-connector was left open or joined to a 7-stage cascade impactor (Intox, Albuquerque, NM). The horizontal outlet of the second T-connector remained open throughout the experiments. Air was drawn by the house vacuum through the impactor at a rate of 500 ml/min which was continuously monitored by a calibrated flow meter.

The nebulization experiments were conducted in incremental phases. The first phase lasted 200 s, the second phase lasted 100–400 s during which aerosol particles were sampled with the cascade impactor, the third phase lasted 300 s, the fourth phase lasted 100–400 s and again the particles were sampled with the impactor, and the fifth and final phase consisted of running the nebulizer until it began to sputter. Aliquots of the solution in the nebulizer chamber were initially taken as well as after each phase of running. The aliquots of the initial solution served as standards. The mass of the solution in the nebulizer was also measured initially, after each phase, and after aliquots were taken. At the end of the experiment, aliquots from the nebulizer were taken, and all T-connectors and tubing were rinsed. The rinse solution was water for solutions containing fluorescein and PVA and 20% water in ethanol for solutions containing BDP. The aliquots and rinses were diluted with water or the ethanol solution so that the measured absorbance of the samples and aliquots fell within the linear portion of the standard curves.

The formulations that were nebulized were: fluorescein in water, fluorescein in 2.5% PVA, 2 mg/ml BDP as NanoCrystals™ in 2.5% PVA with and without fluorescein, and 2 mg/ml BDP as micronized suspension in 2.5% PVA with and without fluorescein. An accurately weighed mass of about 5 g of solution/dispersion was nebulized in each experiment. The absorbance of fluorescein was determined at 490 and 485 nm in the water and ethanol/water solution, respectively. The same stock solution was used for standards and filling the nebulizer and the same ethanol solution was used as a diluent for all samples taken. There was no interference from either BDP or PVA at the concentrations used in the measurement of the absorbance of fluorescein. For BDP, the absorbance was measured at 240 nm in formulations that did not contain fluorescein.

The total (liquid and vapor) output concentration, TVOC, in microliters of liquid per liter of air was calculated from the gravimetric changes in the nebulizer, the specific gravity, ρ, the time of nebulization, t, and the air flow rate, Q_a, as follows (13):

$$TVOC = [(M_f - M_i)/\rho Q_a t]$$

where M is the mass and the subscripts, i and f, refer to the initial and final states. The total liquid output concentration, TLOC, in microliters of liquid per liter of air was calculated by the following formula (13):

$$TLOC = [(M_f - M_i)/\rho Q_a t][Ln(C_f/C_i)/Ln(V_f/V_i)]$$

where C_f and C_i are the final and initial concentrations of dispersed drug or fluorescein in the nebulizer. The total mass output fraction of fluorescein or BDP was calculated from the assayed mass collected on the impactor and the calculated total mass of solute which was nebulized. The former value was corrected by a factor of 12 because only 0.5 lpm of air passed through the impactor while the total air flow rate through the nebulizer was 6 lpm. The total mass of solute nebulized was calculated from the total mass loss from the nebulizer and the arithmetic average of the initial and final measured concentrations in the nebulizer. The respirable fraction was determined in a similar manner except that only the mass collected on stages of the impactor with a cutoff of less than 3.11 μm was used. The mass median aerodynamic diameter and geometric standard deviation of the particle distribution were obtained by plotting the cumulative mass found on the stages of the impactor as a function of the logarithm of the impactor stage cut-off diameter.

Scanning Electron Microscopy

SEM was performed on the NanoCrystal™ dispersion after nebulization by collecting the particles on 2 cm rectangular glass microscope slides which were placed on every stage of the impactor. The glass slides were removed and sputtered with platinum. Micrographs were obtained with a JEOL 840-II ElectroScan Environmental ESEM (Peabody, Mass.).

RESULTS AND DISCUSSION

This is the first study that explores the potential of NanoCrystal™ dispersions as a formulation technique for poorly water soluble compounds intended for respiratory drug delivery. Beclomethasone dipropionate was chosen as the model compound because of its poor water solubility which makes it a suitable candidate for NanoCrystal™ technology. While jet nebulization is not often the preferred method for generating aerosols, it is the least technically complicated method and allows direct atomization of the formulation (14). Moreover, the use of PVA as a stabilizer needs further study for respiratory drug delivery because it is not biodegradable. Nevertheless, other stabilizers are available for the production of NanoCrystals™ which ultimately may provide an advantage for treatment of respiratory diseases such as asthma and tuberculosis.

The focus of the study was an evaluation of the nebulization process of BDP as NanoCrystals™. In supporting studies, nebulization of water and PVA provided a standard for comparing the Salter nebulizer with other nebulizers and an examination of the effect of this polymer on the output concentration. Nebulization of fluorescein in the presence of NanoCrystals™ and micronized BDP provided a means to determine the effect of these solids on the liquid output concentration. Nebulization of NanoCrystal™ BDP demonstrated the potential of NanoCrystals™ in aerosol formulations. Finally, SEM provided a visualization of the physical stability of the NanoCrystal™ dispersion undergoing nebulization.

The unmilled beclomethasone dipropionate had a mean particle size of 10.5 μm. After milling, the NanoCrystal™ dispersion of beclomethasone dipropionate in 2.5% polyvinyl alcohol had a mean particle size of 267 ± 84 nm. This size remained constant throughout the course of the study, and following 7 months storage at room temperature, the mean size was found to be 282 ± 73 nm. The specific gravity of 2.5% PVA was found to be 1.0015 which was used with the density of water at 22°C to convert the measured mass to volume when necessary.

The relative viscosity was 2.7542 ± 0.0052 (mean ± SD). The solubility of BDP was estimated to be 0.18 ± 0.018 mg/ml which was at the limit of detection for the UV determination. The addition of 2.5% PVA caused a slight increase in the amount of BDP in solution. Since the concentration of BDP in the nebulization experiments was 2 mg/ml, the amount of BDP in solution was neglected.

The process of jet nebulization involves the passage of air through a small tube which draws liquid from a reservoir (13–15). The shearing force of the air past the liquid draw tube results in the formation of aerosol particles. These particles are directed towards a baffle where the larger particles impact and can undergo further atomization while the remaining solution is returned to the reservoir. The passage of air through the device causes evaporation, and thus volatile solvents have larger outputs than nonvolatile components. The liquid and vapor contributions to the output may be estimated by measuring the change in concentration of a nonvolatile component in the reservoir (13–15).

In Figure 1, the total output concentration and the liquid output concentration, expressed as microliters per liter of air, are provided for the four formulations: fluorescein in water, fluorescein in 2.5% PVA, fluorescein in 2.5% PVA containing 2 mg/ml BDP NanoCrystals™, and fluorescein in 2.5% PVA containing 2 mg/ml micronized BDP. The total output concentration is the volume of formulation per volume of air that leaves the nebulizer in the form of liquid aerosol droplets and in the form of vapor. The liquid output concentration differs in that it is the volume of formulation per volume of air that leaves the nebulizer in liquid aerosol droplets. It is the latter quantity that is of interest, since only the liquid droplets can carry drug.

The initial total output concentration for water exceeded 50 μl/l which is typical of jet nebulizers. Although the output concentration fell rapidly, the total time to empty the nebulizer was about 900 s or 15 min. The addition of 2.5% PVA caused a significant decrease in the output concentration to about 30 μl/l. However, the output concentrations determined at later times were comparable to that observed with water. The relative viscosity of the 2.5% PVA solution was 2.75 which could cause a decrease in the output concentration (16). The addition of BDP NanoCrystals™ and micronized BDP did not have any effect on the output concentration of the nebulizer or on the length of time to empty the nebulizer (Data not shown). The concentration of BDP was 2 mg/ml, thus no effect on the nebulizer performance would be expected from this low concentration of particles.

In Figure 1b, the liquid output concentration is given as a function of time for the nebulization of the four formulations. For water, the liquid output concentration was initially high but fell rapidly with time. From a comparison of Figure 1a and 1b, it is evident that the majority of the output concentration arises from the liquid aerosols, and evaporation has a minor role with water. With 2.5% PVA, the initial liquid output concentration was lower in comparison to that obtained with water. However, the liquid output concentration in the presence and absence of PVA at later times was similar. In contrast to the results with water, evaporation was a significant portion of the total liquid output when PVA was present. Finally, BDP as NanoCrystals™ or as a micronized suspension had no detectable effect on the liquid output concentrations of the PVA solution.

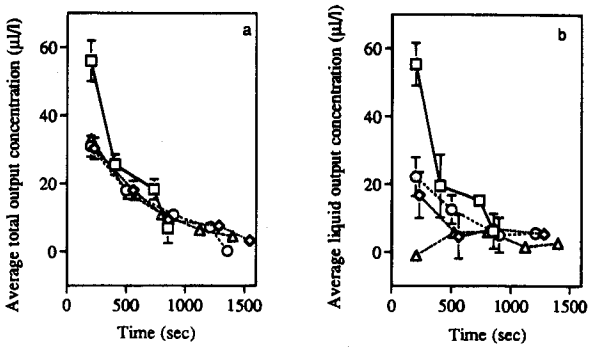

Fig. 1. (a) Total volume output concentration and (b) total liquid output concentration for formulations of (□) fluorescein in water, (◇) fluorescein in 2.5% PVA, (○) fluorescein in 2.5% PVA with 2 mg/ml BDP NanoCrystals™, and (△) fluorescein in 2.5% PVA with 2 mg/ml micronized BDP.

The continuous fall in liquid output concentration presumably arises from the continuous changes in the properties of the dispersion in the nebulizer chamber (13–15). For water, the fall in output has been attributed to the decrease in temperature which in turn increases the surface tension and viscosity. While a similar argument can be made for the nebulization of solutions containing PVA, evaporation also causes a continual increase in the concentration of PVA. This would lead to a progressively more viscous solution which theoretically would further decrease the output (16).

Table I contains a summary of the total mass output fractions of solute and respirable mass output fractions of solute. The total output fraction is the ratio of the output determined by the impactor and the output determined by the change in mass and concentration in the nebulizer. Two collection periods were used, and there was no difference between the two sets. Thus, Table I represents the pooled averages for both collection periods of three different runs. Also note that the T-connectors and tubing contained less than 5% of the initial amount of fluorescein or BDP placed in the nebulizer. Since the total output was determined only during the impactor collection times, the output does not necessarily represent that which would be obtained from nebulization of the entire volume in the nebulizer.

Moreover, the impactor sampled only a portion of the aerosol cloud and thus the results may not reflect the properties of the entire aerosol dispersion. While probably useful for comparison purposes, concerns of nonisokinetic sampling remain. Nevertheless, other studies with an Anderson Mark I impactor which allowed collection of the entire aerosol cloud indicated that NanoCrystal™ formulations had a smaller particle size distribution in comparison to micronized BDP formulations, but respirability could not be estimated due to evaporation during particle collection. The Intox impactor had the advantage of not requiring ancillary air flow, and therefore the particle size was not altered by additional evaporation.

The output fraction of fluorescein atomized in water was 0.63. Losses occurred due to impaction of aerosol particles on the lid of the nebulizer which did not return to the reservoir. The respirable mass fraction was only 0.11 which is relatively small. The respirable fraction was determined in a similar manner to the total output fraction, but only the mass collected on stages with a cutoff diameter less than or equal to 3.13 μm

Table I. Total Output and Respirable Fractions of Fluorescein and BDP Calculated from the Intervals Where Particles Were Collected by the Cascade Impactor (mean ± sd, n = 3)

Formulation	Total Fluorescein output fraction	Total BDP output fraction	Respirable Fluorescein output fraction	Respirable BDP output fraction
Water	0.630 ± 0.111	—	0.110 ± 0.011	—
2.5% PVA control	0.371 ± 0.060	—	0.123 ± 0.033	—
2.5% PVA + NanoCrystals™	0.324 ± 0.086	0.401 ± 0.097	0.097 ± 0.020	0.141 ± 0.020
2.5 % PVA + Micronized BDP	0.367 ± 0.097	0.138 ± 0.070	0.119 ± 0.019	0.0274 ± 0.018

were used in the calculation. While this represents an arbitrary means of defining particle size, it provides a means to compare the particle size distributions which were not amenable to the typical analyses used in characterizing particle size distributions. While other nebulizers have shown much better output fractions as well as respirable fractions (15), the formulation did not foam in the Salter nebulizer. In preliminary studies, a number of other nebulizers were tested, but a considerable amount of foam was produced that moved up the T-connector and interfered with the aerosol collection process. The conical assembly of the nebulizer chamber appears favorable for the nebulization of PVA solution.

The addition of PVA resulted in a reduction in the output fraction of fluorescein to 0.37 where more material adhering to the nebulizer lid was evident. The addition of NanoCrystals™ or micronized BDP did not significantly change the output fraction. These were found to be 0.32 and 0.36, respectively. The output fractions of BDP were also determined from NanoCrystal™ and micronized BDP formulations in the absence of fluorescein as shown in Table I. For the NanoCrystal™ formulation, the output fraction was 0.40 which is similar to the fraction obtained with fluorescein. Therefore, the NanoCrystal™ formulation has an output fraction which is equivalent to that obtained with a water soluble compound. However, the output fraction obtained with micronized BDP was only 0.14 which is only about one third of that found with fluorescein. Thus, considerable disproportionation occurred with the micronized suspension of BDP.

For the respirable output fractions, similar results to the total fractions were seen. The addition of 2.5% PVA resulted in a respirable fraction of fluorescein of 0.12 which is comparable to the 0.11 that was observed with water. Thus, although PVA did reduce the total output fraction, there was no significant effect on the respirable fraction of fluorescein. The respirable fraction of fluorescein, when NanoCrystals™ were included in the formulation, was 0.097 whereas the respirable fraction of BDP was 0.14. There was no statistically significant difference between these numbers at 95% confidence. Thus, the respirable fraction of BDP when formulated as a NanoCrystal™ dispersion is equivalent to that obtained for a water soluble compound. This finding is consistent with the theoretical work of Gonda and co-workers (10,11). Moreover, the aerosol particles would have to be less than 1 μm in order for the NanoCrystals™ to undergo theoretical disproportionation. For the micronized suspension, the respirable output fraction of BPD was 0.027. Not surprisingly, the BDP suspension with a average particle size of 10 μm is not efficiently nebulized into 3 μm respirable aerosol particles.

While it is typical that the mass median aerodynamic diameter and geometric standard deviation be provided, these do not have much significance for this study. Plots of the cumulative fraction as a function of size on linear or logarithmic scales did not yield a linear relationship for any of the formulations tested. Rather, it appeared bimodal. Moreover, much of the mass was collected on the top stage which creates additional difficulties in interpreting the results beyond analysis of the respirable fraction.

The final study was a examination of the NanoCrystals™ after they had been subjected to the process of nebulization. Scanning electron microscopy was conducted of aerosol particles deposited on the impactor from the 2.5% NanoCrystal™ dispersion. NanoCrystals™ were spherically shaped which is similar to their appearance before nebulization (Figure 3). It appears that the NanoCrystal™ particles remain physically stable during the course of nebulization.

Many existing formulations intended for aerosol delivery are solutions, although there are a few which are solid dispersions in propellants (1). The reason lies in the great difficulty in preparing solid-in-liquid dispersions which will deliver particles of a respirable size. In considering the energetics, there is a linear increase in surface energy with a decrease in the mean

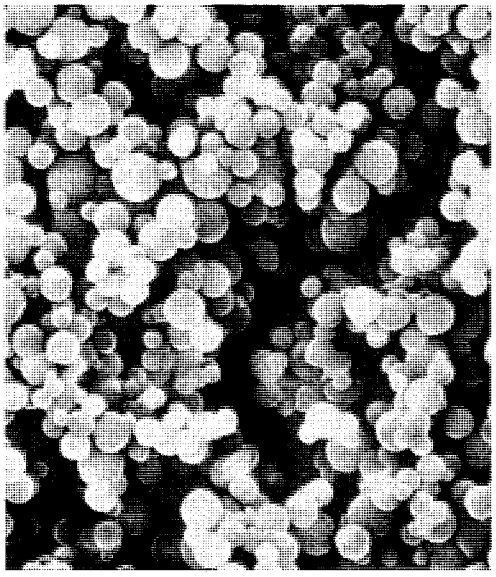

Fig. 2. Scanning electron micrographs of NanoCrystals™ collected by the cascade impactor after being aerosolized from a 2.5% PVA dispersion (×5,000).

radius of the dispersion. Moreover, at particle sizes less than about 5 μm, there is an additional term arising from the particle curvature. This latter effect causes Ostwald ripening which can quickly destroy the respirability of micronize drug suspensions. Herein lies the great potential of NanoCrystals™. These solid-in-liquid dispersions are stabilized by a reduction in the surface energy as well as by steric and/or charge repulsion depending on the choice of stabilizers. Thus, respirable dispersions can not only be readily produced but also exhibit stability for acceptably long shelf lives.

A number of alternatives to formulating poorly water soluble drugs in aqueous dispersions for respiratory drug delivery have been suggested. These include solubilization in liposomes or incorporation into microemulsions or microparticles (17). The NanoCrystal™ approach is unique by formulating the drug in the solid state. This may have distinct advantages with respect to chemical stability and insensitivity to composition perturbations.

CONCLUSIONS

A physically stable NanoCrystal™ dispersion of beclomethasone propionate has been prepared in an aqueous medium and has been shown to be suitable for nebulization. The nebulization process appeared to be dominated by the properties of the aqueous medium and insensitive to the presence of NanoCrystals™. A nebulized aqueous dispersion of NanoCrystals™ of beclomethasone propionate has a greater fraction of respirable drug in comparison to the nebulized micronized suspension of beclomethasone propionate. This suggests that with formulation optimization, NanoCrystal™ dispersions can offer an efficient method of respiratory drug delivery for poorly water soluble compounds.

ACKNOWLEDGMENTS

We acknowledge the helpful discussions with Dr. William Bosch. Excellent technical support was provided by Marina Baum. We thank Dr. Yen-Lane Chen for performing the scanning electron microscopy.

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