Note: Dissolution of Aerosol Particles of Budesonide In Survanta™, a Model Lung Surfactant

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ABSTRACT: The effect of a pulmonary surfactant extract from bovine lung, Survanta™, on the dissolution rate of aerosol particles of budesonide was determined. Aerosol particles of budesonide were generated from an ethanol solution, dried, and collected by a cascade impactor for characterization or by a liquid impinger for dissolution experiments. Powder x-ray diffraction, differential scanning calorimetry, differential thermal analysis, and scanning electron microscopy were used to characterize the aerosol particles and starting material. No change in phase was detected, although the aerosol particles appeared to contain residual solvent. The dissolution rate of the aerosol particles in saline was low and variable. Survanta™ increased the extent of dissolution of budesonide in proportion to the added concentration, which was also verified by equilibrium solubilization studies. Survanta™ also increased rate of dissolution, in a manner similar to sodium dodecyl sulfate. Analysis of the concentration of budesonide following ultracentrifugation indicated that there is rapid equilibration of budesonide between the Survanta™ and aqueous phase. These results show that lung surfactant has the potential of enhancing the rate and extent of dissolution of drugs administered to the lung. © 2001 Wiley-Liss, Inc. and the American Pharmaceutical Association J Pharm Sci 90: 98–104, 2001

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INTRODUCTION

There is a growing interest in the delivery of drugs to the respiratory tract because of the recent technological advances in devices that produce respirable aerosols. These advances have been possible because of the relatively good understanding of the factors that influence respiratory deposition.[1] In addition to being selectively deposited within the respiratory tract, drug particles must also distribute to the site of action. For the latter to occur, the drug must first dissolve and then be transported into or through the cells of the lung.

Presently, the majority of drugs intended for deposition in the lung are formulated as microcrystalline solids, and thus there often is a requirement for dissolution. Drug particles delivered to the periphery of the lung undergo dissolution, but may also be engulfed by macrophages. Drug particles delivered to the tracheal/bronchial regions of the lung undergo dissolution as well but can also be cleared from the lung at a relatively rapid rate by the mucociliary system and ultimately arrive in the gastrointestinal tract. Because of the limited amount of aqueous fluid available for dissolution of the drug in the lung, the wetting and dissolution of aerosol particles have been suggested to be influenced by the presence of lung surfactant.[2,3] Moreover, lung surfactant was suggested as being a key factor in determining the relative long retention in the lung for the amphipathic peptide, detirelix.[2] Hochhaus et al. have provided a series of studies in which retention of triamcinolone acetonide in the lung was sought.[4–6] Although the incorporation of the steroid within liposomal formulations became the research focus, the blood concentration profile obtained from intratracheal instillation of a suspension of triamcinolone was suggested to be influenced by the dissolution rate of the solid par-

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ticles.[4] In our own laboratory, the total amount of drug in solution was found to be increased in the presence of the model surfactant, Survanta™.[7]

In this study, the dissolution of aerosol particles of budesonide in the Survanta™ was determined to test the hypothesis that lung surfactant increases the rate and extent of dissolution of poorly water-soluble drugs. It was found that Survanta™ has a significant effect on the extent of dissolution by increasing the amount of budesonide in solution. Moreover, particle wetting appears also to be substantially facilitated by the presence of surfactant.

EXPERIMENTAL SECTION

Materials

Budesonide and sodium dodecyl sulfate (SDS; 99+ percent, electrophoresis grade) were purchased from Sigma Chemical Company (St. Louis, MO) and used as received. Absolute ethanol was obtained from Aldrich Chemical Company (Milwaukee, WI), and Survanta™ was received from Ross Laboratories (Columbus, OH).

Methods

Aerosol particles of budesonide were obtained by nebulizing an ethanol solution with an ultrasonic nebulizer (Holmes, Milford, MA) operating at a frequency of 2 MHz and entraining the aerosol particles with air at a flow rate of 0.5 liters/min. The aerosol cloud was then passed through a cylindrical charcoal column to remove the ethanol and thereby yield dry, solid aerosol particles.[8,9] The mass output was determined by capturing the aerosol particles with microporous filters, extracting with ethanol, and determining the absorbance at 244 nm (Beckman spectrophotometer, model DU-70, Palo Alto, CA). The concentration was interpolated from appropriate standard calibration curves. The aerosol particles and starting microcrystalline budesonide were evaluated by differential thermal analysis (model 951 thermogravimetric analyzer, DuPont Instruments, Wilmington, DE), melting point apparatus, and X-ray powder diffraction (model D500, Siemens, Madison, WI).

A cascade impactor (Intox, Albuquerque, NM) operating at 0.5 lpm with cutoff diameters of 5.09, 3.13, 1.93, 1.21, 0.77, 0.59, and 0.33 μm was used to monitor the size distribution of the aerosol. The mass median aerodynamic diameter, MMAD, and geometric standard deviation, GSD, were obtained by a linear fit of the cumulative undersized collected mass plotted on a probability scale as a function of logarithm of the cutoff diameter using KaleidaGraph™ (Synergy Software, Reading, PA).[10] The particles were also captured by a 0.2-μm membrane filter (Millipore, Milford, MA) and subjected to scanning electron microscopy (Center for Interfacial Engineering, University of Minnesota, Minneapolis, MN). The particles were coated with gold using vapor deposition and scanned at 10 kV.

The solubility of budesonide in normal saline and the solubilization in SDS and Survanta™ were determined by placing excess microcrystalline budesonide in dialysis bags (MWCO 10,000 Da) along with either saline, 0.01% SDS in saline, or 0.01, 0.025, or 0.05% Survanta™ diluted with saline at 37 °C.[11] The bags were tied and placed into Pyrex™ screw-capped test tubes with Teflon™ liners that contained either saline, SDS, or diluted Survanta™. The tubes were then oscillated for 48 h. The budesonide concentration in the aqueous phase was determined from the outer solution by measuring the absorbance at 244 nm against appropriate standards. The budesonide concentration in the dispersion was measured by first dissolving an aliquot in ethanol, and then measuring the absorbance spectrophotometrically at 244 nm. In addition, aliquots containing Survanta™ were taken and subjected to ultracentrifugation (56,000 × *g,* model J2-21 Beckman, Palo Alto, CA), and the supernatant was assayed for budesonide in a similar manner.

For the study of the dissolution of aerosol particles, an air sampling liquid impinger (tip 30 mm from bottom with an ~125-mL capacity, Ace Glass, Vineland, NJ) was filled with saturated solutions of budesonide in either normal saline solution or 0.01% SDS and placed in an ice bath. After collecting a sufficient mass of aerosol particles in ~30 min, the dissolution process was initiated by diluting the impinger solution in a one-to-six (v/v) ratio with saline or solutions of SDS or Survanta™ held at 37 °C. The solutions were oscillated in scintillation vials on a shaking water bath at 37 ± 0.5 °C.

Aliquots were taken and centrifuged in a tabletop centrifuge at high speed (~1000 × *g,* International Equipment Company, Needham, MA) for 10 min to remove undissolved budesonide particles. An aliquot of the supernatant was dissolved in ethanol, and the absorbance determined at 244 nm against appropriate standards from

which the total concentration of budesonide in solution was calculated. The presence of SDS and Survanta™ did not interfere with the absorbance measurement. The concentration of dissolved budesonide in the aqueous phase in samples containing Survanta™ was determined by subjecting the samples to ultracentrifugation at 56,000 × *g* for 30 min. This procedure caused the sedimentation of both the budesonide particles as well as the Survanta™. Aliquots of the supernatant were taken, and the absorbance measured against appropriate standards. Thus, with these two different centrifugation steps, both the total budesonide and budesonide in the aqueous phase were determined.

RESULTS AND DISCUSSION

As a foundation for analyzing the study of the dissolution in lung surfactant, the properties of the budesonide aerosol particles were characterized. The aerosol particles had a MMAD and GSD of 1 μm and 2.2, respectively. On the scanning electron micrograph, the aerosolized budesonide captured on the membrane filter appeared as spherical solid particles. There was no evidence of interparticle bridging that would be the likely consequence of the capture of wet particles.

Both aerosol and microcrystalline budesonide decomposed with melting in the range 232–237 °C. The powder X-ray diffraction profiles of the two samples were superimposible (Figure 1). However, differential thermal analysis revealed about a 2% weight loss that only occurred with the aerosol particles over the temperature range of 150 to 225 °C (Figure 2). Because both samples have similar X-ray powder diffraction patterns, formation of a solvate was ruled out. It is likely that the solvent is associated with the surface or amorphous regions of the aerosol particles and is not removed under ambient conditions.

The aqueous solubility and solubilization of microcrystalline budesonide were determined to establish baseline measurements for the dissolution study of the aerosol particles. At 0 °C, the solubility of budesonide in 0.01% SDS was 16.1 μg/mL. At a temperature of 37 °C, the value was increased slightly to 20.9 μg/mL (Table 1). In the presence of 0.01% Survanta™ lipid at 37 °C, the total amount of budesonide in solution was 24.4 μg/mL. Further increases in Survanta™ to 0.025 and 0.05% caused the total amount of budesonide in solution to rise to 28.6 and 37.1 μg/mL, respectively. The amount of budesonide solubilized increased linearly with the concentration of surfactant, which is consistent with other studies of the solubilization of drugs by phospholipids.[7,12,13] Because Survanta™ consists of a preparation of 25 mg lipid/mL, on average, 33.6 ± 3.2 μg budesonide were solubilized per milligram of Survanta™ lipid (Table 1).

Budesonide has a reported solubility of 20 μg/mL at 25 °C, which is in good agreement with that obtained in this study.[14] The addition of 0.01% SDS did not affect the amount of budesonide in solution, which is reasonable because this concentration is below the critical micelle concentration of SDS.[15] Because the addition of Survanta™ caused a significant increase in the amount of budesonide in solution of Survanta™, it is of value to compare Survanta™ with native lung surfactant.

Native lung surfactant is secreted by the Type II cells that are located in the alveoli and lower respiratory bronchioles of the lung.[16] It consists of an aqueous dispersion of a mixture of lipids and proteins. The composition is >90% lipid, with the remaining 10% composed of a mixture of proteins. For the lipids, 90% are phospholipids, of which 90% are phosphatidylcholine and another 8–10% are phosphatidylglycerol. It is also of interest to note that the phosphatidylcholines are largely saturated with dipalmitoyl phosphatidylcholine, representing 40% of the phospholipids present. In contrast, the phosphatidylglycerols are enriched with unsaturated lipids.

Survanta™ is a marketed product for surfactant replacement therapy. Survanta™ is produced by enriching the organic-solvent-soluble fraction of calf lung surfactant with synthetic lipids. The reported composition is 11.0–15.5 mg/mL dipalmitoyl phosphatidylcholine (DPPC), 0.5–1.75 mg/mL triglycerides, 1.4–3.5 mg/mL free fatty acids, 0.1–1.0 mg/mL proteins B/C, and 7.65–10.35 mg/mL sodium chloride. Other surfactant replacements are currently used to treat respiratory distress syndrome that are referred to as synthetic replacements because they are not obtained from natural sources. One example is Exosurf, which contains DPPC, hexadecanol, and a synthetic spreading agent, tylaxol. Survanta™ was chosen as the model surfactant for the present solubilization and dissolution studies because it is derived from native surfactant and more closely approximates surfactant in the lung.

The results from the distribution of drug between lung surfactant and an aqueous solution

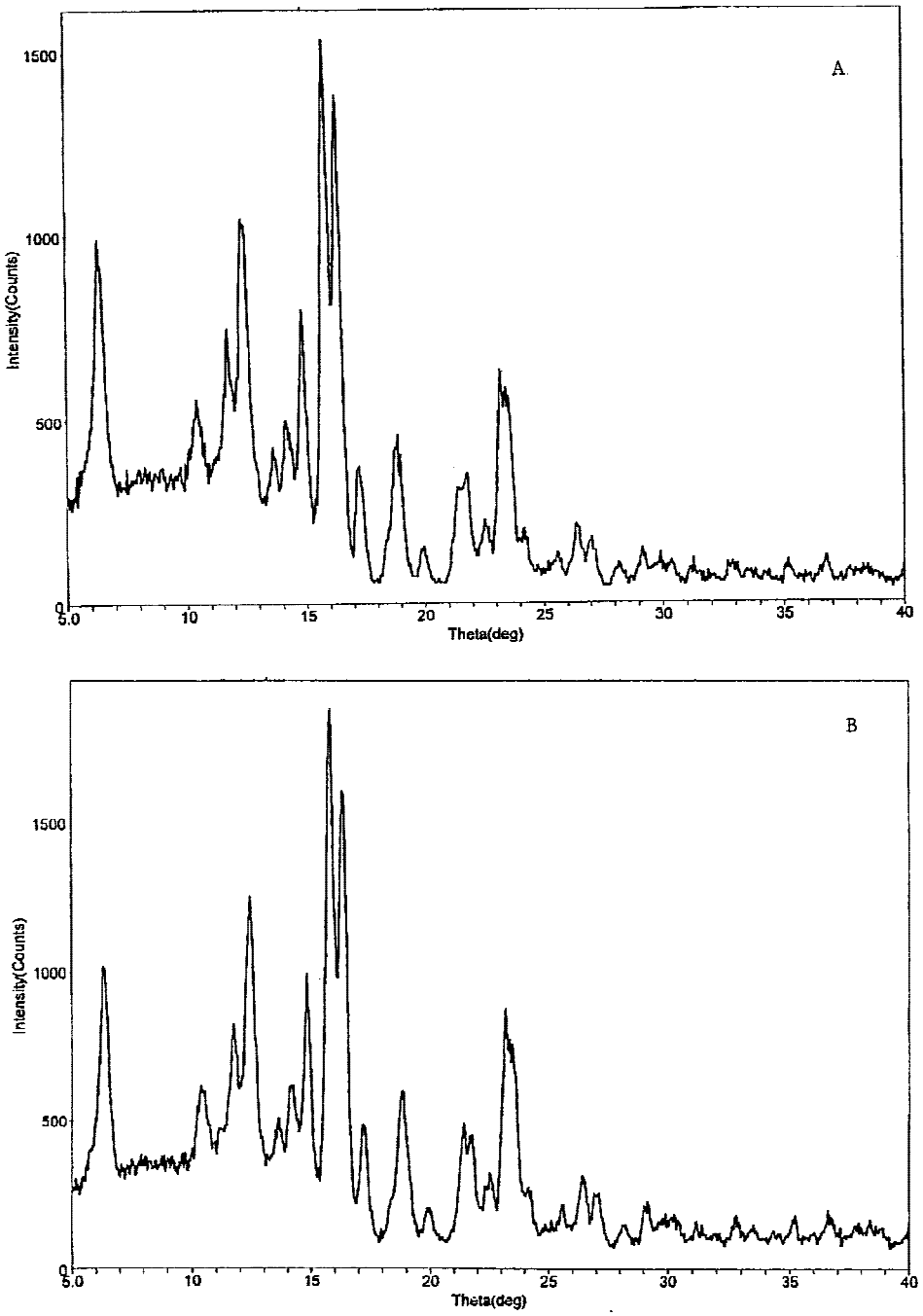

Figure 1. Powder X-ray crystallographic patterns of (A) budesonide as received and (B) aerosols of budesonide.

are consistent with those of an earlier study[7] in which the thermodynamics of transfer of a number of steroids from water to Survanta™ above and below the gel-to-liquid crystalline phase transition were determined. The corresponding Survanta™/aqueous distribution coefficient of budesonide at 37 °C was 1533, which compares well with the results in the published report.

The results from the dissolution studies are given in Figures 3 and 4. In Figure 3, the total amount of budesonide dissolved as a function of time is given. The absolute controls conducted in saline were variable, and a film of budesonide formed at the surface of the impinger during the

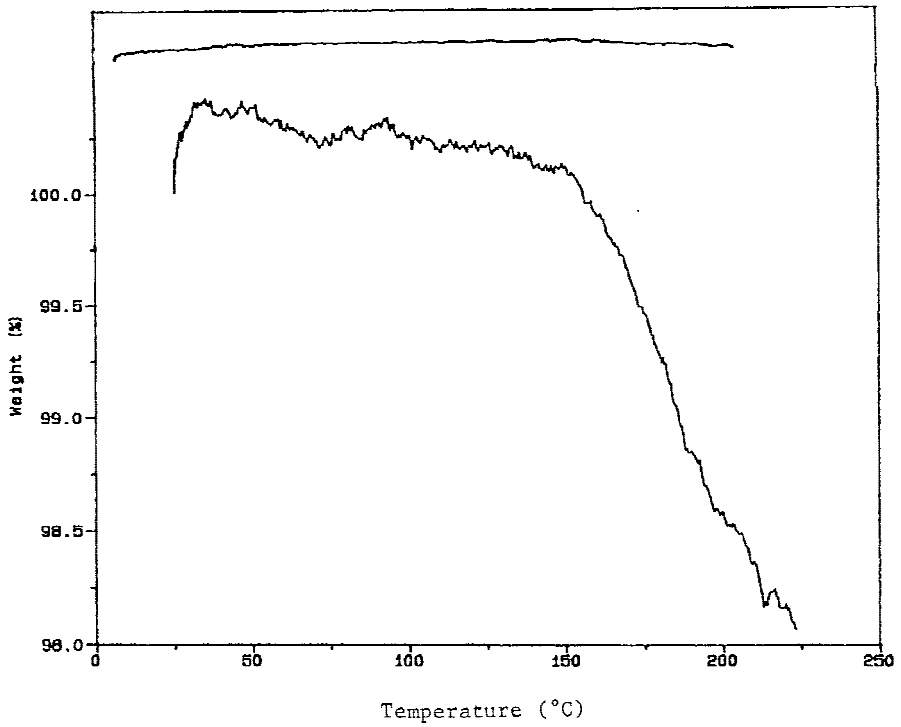

Figure 2. Differential thermal gravimetric scan of (upper curve) budesonide as received and (lower curve) aerosols of budesonide.

collection process. As such, the aliquots taken for dilution for the dissolution experiment were not very reproducible. Nevertheless, it was apparent that after 250 min, the equilibrium solubility was not achieved because the concentration was still below that observed in the solubility study.

To enhance the wetting of the budesonide particles, 0.01% SDS was added to the impinger solution, and a dissolution study was conducted in the presence of 0.01% SDS. In this case, the concentration in solution rose rapidly and a plateau in the concentration was attained in as few as 20 min. However, the presence of 0.01% SDS did not significantly alter the equilibrium solubility of budesonide, which is consistent with the solubilization results.

With the addition of Survanta™ there was also a rapid rise in the concentration of budesonide in solution as a function of time. As with SDS, the equilibrium value was rapidly reached, perhaps in as few as 5 min. Similarly, in keeping with the solubilization results, the final equilibrium amount of dissolved budesonide progressively in-

Table 1. Solubility and Solubilization of Budesonide in Survanta™ at 37°C in Normal Saline[a]

Solution	Solubility
Aqueous	20.9 ± 0.3 μg/mL
0.01% SDS	20.89 ± 0.32 μg/mL
Survanta™ aqueous phase	20.73 ± 0.25 μg/mL
Survanta™ solubilization	33.6 ± 3.2 μg/mg surfactant lipid

[a] Results are expressed as mean ± SD ($n = 3$).

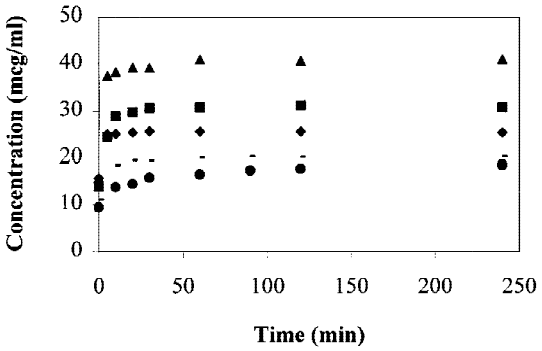

Figure 3. Total concentration of budesonide as a function of time (listed from bottom to top) in aqueous, 0.01% SDS, and 0.01, 0.025, and 0.05% Survanta™ dispersions (percent standard deviation was <10% for aqueous samples and <5% for SDS and Survanta™).

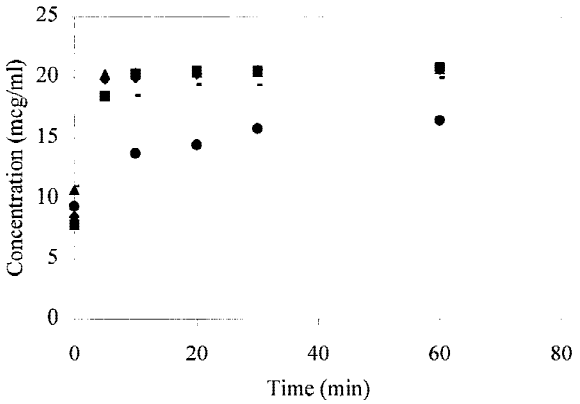

Figure 4. Aqueous concentration of budesonide as a function of time in aqueous, 0.01% SDS, and 0.01, 0.025, and 0.05% Survanta™ dispersions (percent standard deviation was <10% for aqueous samples and <5% for SDS and Survanta™). Symbols same as in Figure 3.

creased with Survanta™ concentration. Moreover, the apparent equilibrium values observed in the dissolution experiments were in very good agreement with those observed in the solubilization experiments.

Because Survanta™ may be removed from the aqueous solution by centrifugation, it was possible to determined the budesonide in the aqueous phase as a function of time. The results from this study are given in Figure 4 along with the SDS control. The concentration in the aqueous phase rose rapidly achieving the equilibrium value in as few as 5 min, similar to the result observed with the total amount of budesonide in solution. However, the final value of the concentration of budesonide in the aqueous phase was independent of Survanta™ concentration and corresponded to the equilibrium solubility. Thus, within the time constraints imposed by the experimental methods, budesonide appeared to maintain an equilibrium distribution between the aqueous and lipid phase of Survanta™.

A number of experimental factors would have a direct impact on the time to reach equilibrium in the dissolution study. Whereas mass of solute, particle size, and volume of solution have a bearing, these were held constant for comparison purposes. In addition, to a first approximation, the equilibrium concentration of budesonide in solution does not affect the rate of dissolution; thus, the only parameter of relevance is the effective surface area of solid available for dissolution. Given the experimental conditions and by using the Hixon–Crowell cube root law, the calculated time to reach equilibrium is <5 min. However, the observed rate was much slower in the saline solution because equilibrium was not attained for >1 h. The plausible explanation for this result is that the poor wetting of the solid led to a decrease in the available surface area for dissolution. Moreover, because the rate increased with SDS and perhaps even more with Survanta™, it would appear that these surface-active agents increased the area available for dissolution. Therefore, lung surfactant can be assumed to have a significant wetting effect on the dissolution of particles deposited in the lung.

In conclusion, the dissolution of aerosol particles has been determined in a model lung surfactant for the first time. It has been shown that lung surfactant has a significant effect on the extent of dissolution and also the rate. The enhanced solubilization appears related to the rich lipid content of the surfactant, and the rate appears to be a consequence of the high surface activity of the lung surfactant. This investigation corroborates the earlier study that showed that the interaction with surfactant promotes wetting of solid particles.[17] In addition, the findings of this investigation provide a quantitative basis for the reports that the pharmacokinetic distribution of drugs is influenced by drug–surfactant interactions.[2,3,18]

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