Process-Induced Crystallinity Changes in Albuterol Sulfate and Its Effect on Powder Physical Stability

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Pharmaceutical powders are often milled to achieve the optimum particle size. These size reduction processes can introduce dislocations and/or defects onto particle surfaces affecting the overall crystallinity of the powder. If enough energy is imparted, amorphous regions on the particle surfaces may be produced. These amorphous regions have the propensity to absorb significant quantities of water. In this study the effect of sorbed water on the physical characteristics of albuterol sulfate is investigated. Physical properties of this compound are studied in both micronized and unmicronized states using scanning electron microscopy, differential scanning calorimetry, powder x-ray diffraction, solution microcalorimetry, laser diffraction particle size analysis and water vapor sorption analysis. Subtle differences in crystallinity induced by air jet micronization are detected by several analytical methods. Amorphous to crystalline conversions are observed, the kinetics of which are found to be both temperature and relative humidity dependent. These experiments show the dynamic nature of micronized albuterol sulfate and aid in the determination of the actual physical state of this pharmaceutical powder.

KEY WORDS: absorption; albuterol sulfate; amorphous; crystallinity; glass transition temperature; moisture; physical stability; water vapor.

INTRODUCTION

Materials used in pharmaceutical preparations are rarely crystallized at the required or optimum size. Consequently, solids are often comminuted at some stage during the manufacture of a dosage form. Significant quantities of energy can be generated during milling and this energy can affect the material being processed in unforeseeable ways. Changes in the physical state of raw materials occurring as a result of particle size reduction are well documented (1–4). The formation of polymorphs or changes in solvation are examples of gross alterations in powder states and have been observed for many drugs. Detection of polymorphs and/or solvates is generally uncomplicated; standard analytical methodology such as differential scanning calorimetry (DSC), thermal gravimetric analysis (TGA), powder x-ray diffraction (PXRD), and infrared spectroscopy, etc., are typically sufficient for the task since these changes often involve a nearly complete conversion between physical states.

Recently, more subtle changes in the physical state of powders have been postulated and observed (5). These changes can be directly related to the energy required for particle size reduction (3,6). During the milling process crystalline disorder can be introduced onto particle surfaces. This disorder, if more extensive than the occasional molecular dislocation, can be viewed as an amorphous region on the surface of the particle (see Figure 1). From a thermodynamic perspective, these amorphous regions are metastable and exist at a higher energy state than the crystalline regions within the particle. Given the proper environmental conditions, in time this amorphous material will convert to the crystalline state.

Zografi and coworkers have contributed significantly to the general understanding of these transitions, particularly with respect to the role of water and its ability to act as a plastisizer when incorporated into amorphous regions of a given solid (7–11). Additionally, important work with regard to methodology for the assessment of crystalline disorder has been advanced by their laboratory. Much of this groundbreaking work has focused on highly amorphous solids, with model compounds comprising of lyophilized sugars and polymeric compounds such as PVP.

In contrast to polymeric, lyophilized or spray dried materials, amorphous character in highly crystalline, micronized powders can be difficult to detect. Since the amount of this material present in a given sample may be very small it is often beyond the limits of detection for traditional analytical methodologies. However, by using a variety of techniques, these subtle, but significant, changes in powder properties may be exposed.

Previously, little work has been done to characterize low levels of amorphous content in predominantly crystalline, air jet micronized pharmaceutical particles (< 3μm diameter). Exhaustive efforts are often required to produce particles of this minute size; typically for use in aerosol applications. This comminution technique requires a tremendous energy input. Despite the rigorous conditions to which these particles are subjected during size reduction, the resulting powders are generally highly crystalline, with amorphous regions comprising only a very small percentage of the mass of the particle. Nevertheless, even small amounts of amorphous material can have profound physical effects. If the conversion process from an amorphous to crystalline state is not well understood and occurs in an uncontrolled manner, it can lead to significant physical stability problems during dosage form development.

Albuterol sulfate is widely used in several commercial inhalation devices. Any changes in the physical state of this powder could have significant consequences with respect to performance characteristics of both metered dose and dry powder inhalation delivery systems. Therefore, the objectives of this research are to (1) compare various conventional analytical techniques for the determination of process induced crystalline changes using albuterol sulfate as a model compound; and (2) utilize multiple analytical techniques to study the dynamics of the amorphous to crystalline conversion and its effect on the physical properties of albuterol sulfate.

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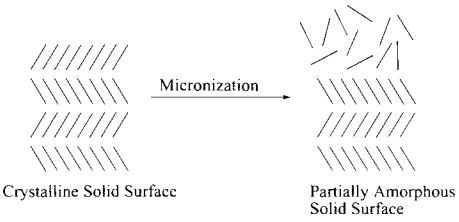

Figure 1. Schematic representation of a crystalline surface (first few molecular layers) before and after micronization. Note how the order of the regularly packed molecules is disrupted after milling.

EXPERIMENTAL

Materials

Albuterol sulfate (Figure 2), a water soluble, nonhygroscopic β_2-agonist was used as received from ICFI (Milano, Italy). This material was air jet micronized using both the equipment and technique described below. Various physical properties of both micronized and unmicronized albuterol sulfate were analyzed initially and after 24 hours storage at 40°C/75% RH (relative humidity).

Methods

Air Jet Micronization (Sturdevant 4″ Mill). The mass median diameter (MMD) of unmicronized albuterol sulfate was approximately 16 μm. Flow rate, feed and grind air pressures and drug feed rate were adjusted such that material was produced with an MMD of 1.5 μm. Both micronized and unmicronized drug were stored at 25°C under vacuum prior to analysis.

Laser Diffraction Particle Size Analysis (LD) (Malvern, Model 2600). Samples were dispersed in a liquid media comprised of 0.7% w/w sorbitan trioleate in trichlorotrifluoroethane. This dispersion was added dropwise to a static measurement cell until an obscuration of 0.25 was reached. Particle size distributions were then collected in triplicate and average values reported.

Scanning Electron Microscopy (SEM) (JEOL, Model 820). Samples were mounted on an aluminum stub and sputter coated with gold/palladium. All micrographs were taken using 15 kV at a viewing angle normal to the stub surface. All micrographs are the product of secondary electron imaging used for surface morphology identification at 5,000× magnification.

Powder X-Ray Diffraction (PXRD) (Philips Vertical Goniometer, copper Kα radiation, proportional detector, diffracted beam monochrometer). Samples were placed in aluminum holders and scanned isothermally at 25°C. The data were collected with an angular range between 5 and 55 degrees 2θ , using a step size of 0.04 degrees/step, 4 seconds/step.

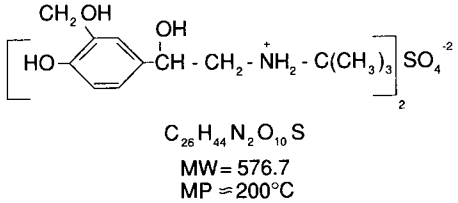

Figure 2. Molecular structure of albuterol sulfate.

Differential Scanning Calorimetry (DSC) (Seiko Instruments, Model 220C). Samples were weighed into aluminum pans (≈ 10 mg) and hermetically sealed. A heating rate of 10°C/min was used over various temperature ranges. All samples were analyzed in triplicate.

Solution Microcalorimetry (SMC) (Setaram, Model C80D). The sample (50 mg) was placed in the lower compartment of the cell and water (3 ml, de-ionized) in the upper compartment. The water and drug are separated by a lid covered with a mercury seal to insure complete separation of the solution components.

The cell was placed in the calorimeter and allowed to thermally equilibrate (approximately one hour). After the baseline was established, the microcalorimeter was inverted three times allowing water and sample to mix. Data was collected in intervals of one second, isothermally at 30°C with a powder amplification of 0.25 mV. All samples were analyzed in triplicate.

Water Vapor Sorption Analysis (WVSA) (VTI, Model MB300G). Samples were placed on the microbalance pan, surrounded by a thermal jacket used for controlled isothermal scanning. All samples were dried under vacuum ($\approx 10^{-6}$ torr) at 60°C until equilibrium was reached (i.e., no more than 5 μg weight change in 5 minutes – three consecutive measurements). Water vapor was then introduced to the sample in increments of 5% relative humidity. All moisture levels were maintained until the sample reached gravimetric equilibrium, as described above. Percent weight change, relative pressure and time data were recorded simultaneously throughout each sample run. All samples were analyzed in triplicate.

RESULTS AND DISCUSSION

Laser Diffraction

The particle size of both micronized and unmicronized samples was determined by LD, the results of which are shown in Table I, reported as the average of the mass median diameters (MMD). The distributions are essentially log normal with a ten-fold difference in MMD between micronized and unmicronized samples. Material stored 24 hours at 40°C/75% RH showed dramatic changes from the initial vacuum-stored micronized albuterol sulfate, with the most significant changes occurring in particles less than 2μm in diameter. However, the samples stored 24 hours at 40°C/75% RH gave

Table I. Malvern Laser Diffraction Particle Size Results[a]

	Unmicronized RT/vacuum	Micronized RT/vacuum	Micronized 40/75-24 Hrs
% Under 5 μm	11	98	73
% Under 2 μm	0	72	6
% Under 1 μm	0	24	1
Mass median diameter D[v, 0.5]	15.7	1.5	3.9

[a] All data are averages of three replicates.

more variable results (≈25% RSD) than the micronized and unmicronized material analyzed initially.

Scanning Electron Microscopy

Figure 3 shows representative SEMs of the unmicronized (top) and micronized (middle) samples at a magnification of 5,000×. These data confirm both the LD data as well as the effectiveness of the micronization process. Significant differences in both morphology and aspect ratios are apparent. Figure 3 (bottom) shows an SEM of micronized material stored 24 hours at 40°C/75% RH. Note the changes in surface morphology of the micronized sample as compared to the initial samples; the crystal edges are more rounded and smooth and significant interparticle bridging appears to have occurred during storage.

In addition, for the micronized 40°C/75% RH sample, most of the submicron-sized particles have fused to the larger particles. The unmicronized sample, also exposed 24 hours at 40°C/75% RH, exhibited no significant changes in morphology (photomicrograph not shown).

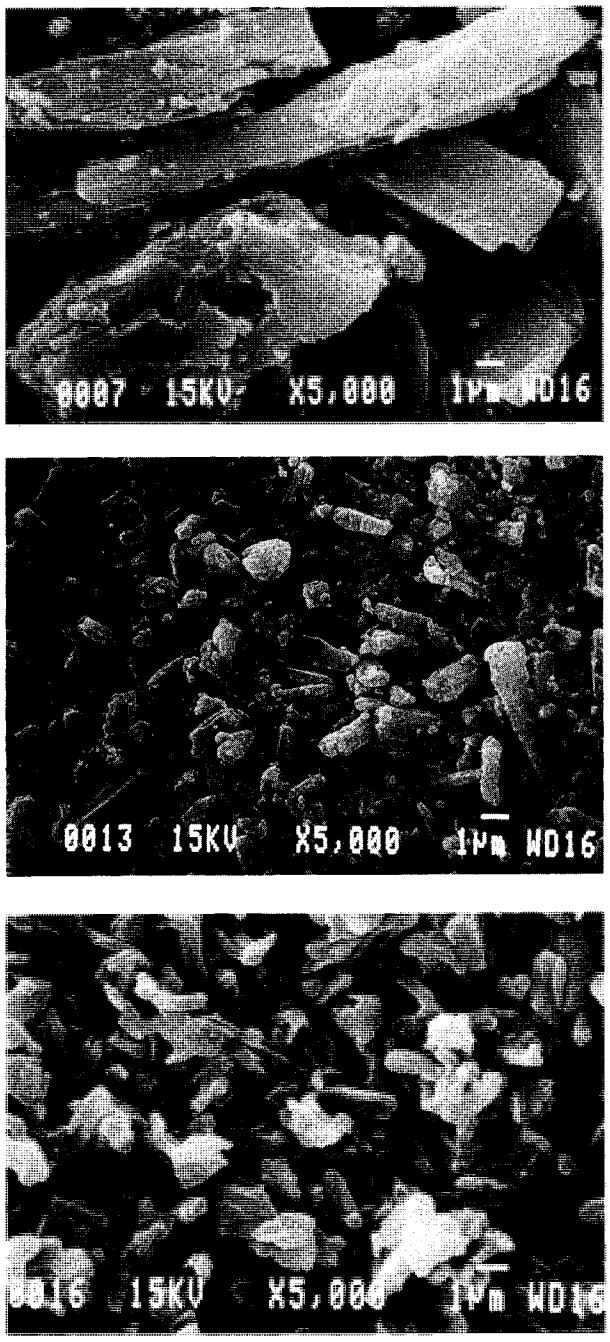

Figure 3. Scanning electron micrographs of albuterol sulfate: unmicronized (top), vacuum-stored micronized (middle) and micronized stored 24 hrs at 40°C/75% RH (bottom). Photographs taken at a magnification of 5,000×. Bar at lower right is 1 µm in length.

Powder X-Ray Diffraction

Powder X-ray diffraction, one of the more widely used methods for crystallinity quantitation, did not have the sensitivity required to conclusively distinguish between the micronized and unmicronized samples investigated in this study, nor was it useful in detecting changes in micronized powders after storage at various environmental conditions. The results of the PXRD analysis (see Figure 4 for representative scans) do show small differences in peak intensity, however, these are most likely due to preferred orientation; note the aspect ratio of unmicronized sample from Figure 3. While there was some loss of peak detail at the higher angles for the micronized sample, this phenomena was not always reproducible. No significant differences in either peak position or band broadening (indicators of crystalline physical state) were observed between the micronized and unmicronized powders nor were any major differences observed between the initial micronized material and that stored 24 hours at 40°C/75% RH. These results are not surprising, since detection of amorphous content in highly crystalline organic powders typically becomes difficult at levels below 10% when using this technique.

Differential Scanning Calorimetry

Differences in crystallinity between micronized and un-

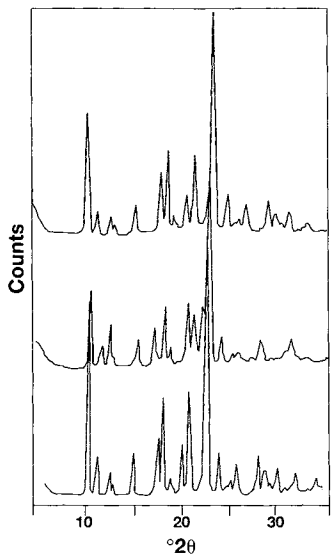

Figure 4. Powder X-ray diffraction scans of albuterol sulfate: unmicronized (bottom), vacuum-stored micronized (middle) and micronized stored 24 hrs at 40°C/75% RH (top).

micronized albuterol sulfate were investigated nonisothermally using DSC. This technique proved to be much more sensitive than PXRD in its ability to detect differences between micronized and unmicronized albuterol sulfate. The data for both micronized and unmicronized samples are shown in the top panel of Figure 5 (representative scans). Both samples melt with degradation near 200°C. The unmicronized material shows no significant thermal events prior to melting. However, a very small exothermic peak is observed for the micronized sample, occurring at 85°C. By focusing on this region of the DSC tracing and changing the scale (Figure 5, bottom panel), a small endotherm near 64°C can also be observed. A subsequent scan of the identical micronized material showed no thermal events prior to melting, indicating that the premelting thermal events described above are irreversible. Micronized material stored 24 hours at 40°C/75% RH was also examined by DSC. This scan displayed similar characteristics to that of both the unmicronized and twice run micronized samples.

It has been shown by Saleki-Gerhardt et al (5) that the type of DSC profile shown in Figure 5 for the vacuum stored, micronized compound, can be indicative of the presence of small quantities of amorphous material in the solid.

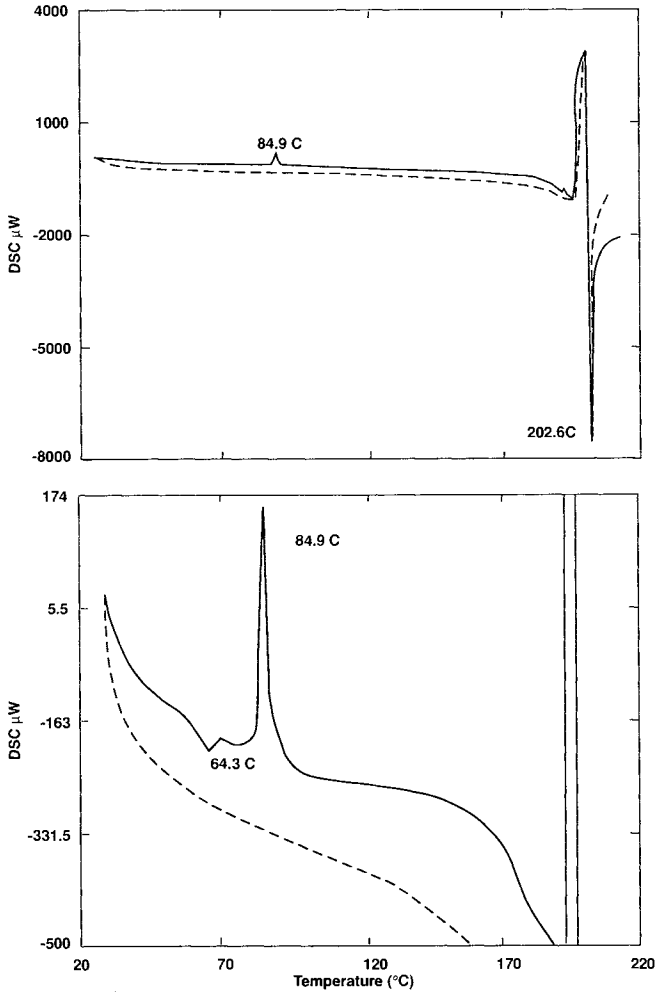

Figure 5. Differential scanning calorimetry scans of albuterol sulfate (solid lines for vacuum-stored micronized, dashed lines for unmicronized). Bottom figure represents same data at a reduced scale.

The glass transition temperature (T_g) of the amorphous material is most likely encompassed in the early endotherm at 64°C. While T_g's usually manifest themselves on DSC scans as pure baseline shifts, it is speculated that, following the glass transition, there is a structuring of the molecules prior to crystallization which results in a release of energy and a return to baseline. This structuring can be viewed as a type of enthalpic annealing (12). In addition, the endotherm at 64°C agrees well with a predicted T_g of 60°C based on the following equation whereby $T_g = 0.7(T_m)$ in degrees Kelvin (10). The exothermic peak at 85°C is consistent with a crystallization event (T_c), associated with the conversion of the amorphous material to a crystalline state. Micronized material stored at 40°C/75% RH showed no early thermal events by DSC, presumably, due to a prior amorphous to crystalline conversion at that environmental condition.

Solution Microcalorimetry

Solution microcalorimetry also proved to be useful in detecting small differences in crystallinity between micronized and unmicronized albuterol sulfate. Figure 6 shows representative isothermal, solution (aqueous) microcalorimetric scans for micronized and unmicronized albuterol sulfate (vacuum stored). All runs indicate exothermic solution behavior; the average $\Delta H_{solution}$ values are -3.67 and -6.01 Cal/gram for the unmicronized and micronized lots, respectively.

Additionally, a heat and moisture conditioned sample (24 hours at 40°C/75% RH) was also measured by SMC resulting in an average $\Delta H_{solution}$ of -0.63 Cal/gram. Significant differences in the solution enthalpy/gram of material are observed. The order, from highest to lowest energy, is micronized > unmicronized > micronized/conditioned. By detecting amorphous material present in albuterol sulfate (by comparison of $\Delta H_{solution}$ values), this method also proved to be predictive of powder instability.

Bystrom and Briggner (13) have shown recently that at little as 1% amorphous content in a powder could be discerned using microcalorimetry. Since PXRD and DSC results suggest that the overall crystallinity differences between micronized and unmicronized samples are less than 10%, the differences detected by SMC are most likely surface energy related. While, it is reasonable to assume that the micronized material would have a higher surface energy than unmicronized material, it is not obvious that the micronized sample stored at 40°C/75% RH would have a lower surface energy than unmicronized material. However, this data can be rationalized if one considers the morphology of these particles. Scanning electron microscopy of the micronized sample exposed to 40°C/75% RH (Figure 3) shows a "rounded" appearance to the crystal surfaces as well as significant interparticiple bridging (due to the crystallization of the amorphous regions of the particle), while the unmicronized sample has well defined crystal faces with sharp edges.

It is reasonable to assume that the energetic order of the various samples given by SMC is correct, based on the SEM results, since the sharp edges of the unmicronized albuterol sulfate crystals contribute to a higher surface energy than the smooth crystal surfaces exhibited by the micronized/40°C/75% RH sample.

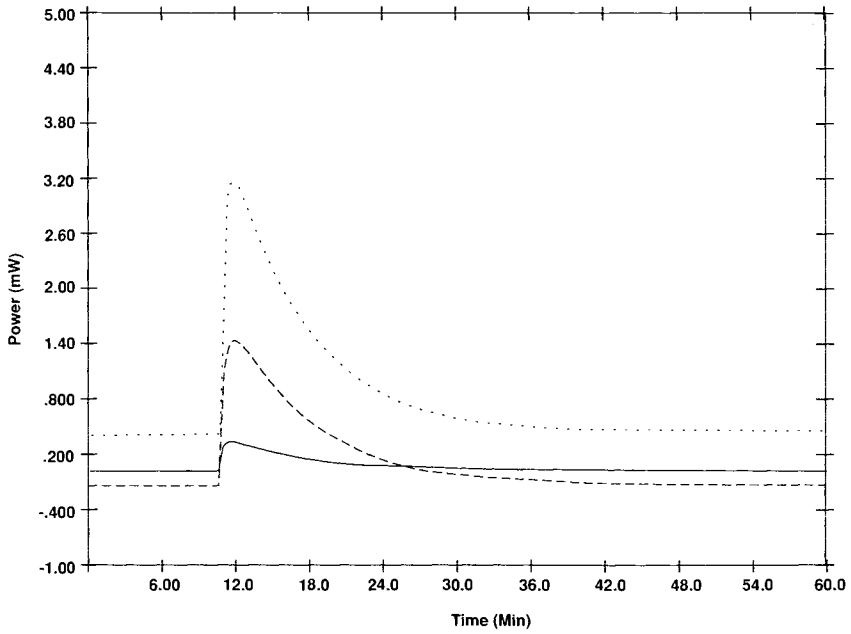

Figure 6. Solution microcalorimetry data for vacuum-stored micronized (dotted), unmicronized (dashed) and 40°C/75% RH-stored micronized (solid) albuterol sulfate.

Water Vapor Sorption Analysis

The data described above suggest that the amorphous to crystalline conversion of albuterol sulfate is mediated by heat and moisture. Therefore, a prudent method by which to study this phenomenon is water vapor sorption analysis. A comparison of the water vapor sorption data for vacuum-stored micronized versus unmicronized samples is shown in Figure 7 (typical scans). Significant differences in the total amount of water sorbed are observed, with the unmicronized material having a maximum value approximately 10 fold greater than the micronized material at 95% RH. This was somewhat unexpected based on the large differences in both particle size and surface area between these two samples. Uptake of such large quantities of water can occur as the result of hydrate formation, deliquescence or capillary condensation. To the authors knowledge, no hydrate forms of albuterol sulfate have been reported.

The crystal structure of albuterol sulfate is known (14),

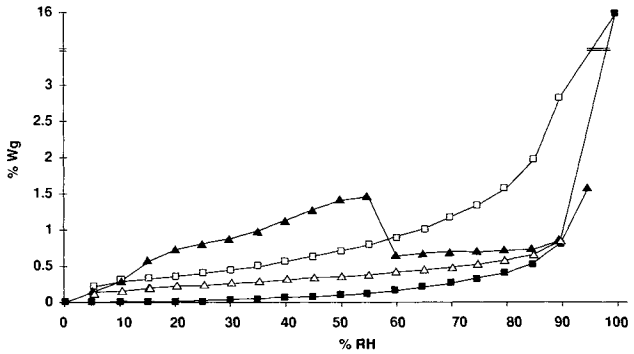

Figure 7. Water vapor sorption data for unmicronized and vacuum-stored micronized albuterol sulfate (▲—Sorption/Micronized, △—Desorption/Micronized, ■—Sorption/Unmicronized, □—Desorption/Unmicronized). Note the break in the scale on the y-axis.

and there does appear to be room for water within the lattice which could account for the water sorption observed. However, one would expect that water could also enter the lattice of the micronized material as well (provided that the amorphous material does not act as a barrier), and this material had minimal moisture uptake. Since deliquescence would be expected to occur for both the micronized and unmicronized powders as well, and this was not observed, this mechanism is unlikely. However, as demonstrated previously, there are significant morphological differences between micronized and unmicronized albuterol sulfate. Close examination of the SEMs (see Figure 3) reveals numerous cracks and fissures on the unmicronized material which are absent from the micronized material. These regions could be susceptible to moisture condensation. In addition, the hysteresis observed for the unmicronized material at high relative humidities is consistent with a capillary condensation mechanism. While the true nature of this phenomenon eludes us at present, this is certainly an interesting phenomenon which warrants further work.

In addition to the differences in overall moisture uptake, at a critical relative humidity (50%) a dramatic weight loss (≈ 50% of the maximum uptake) is observed for the micronized sample during the absorption phase. It is believed that this water loss is also indicative of an amorphous to crystalline transition.

It is well known that water can readily enter amorphous regions of solids and act as a plasticizing agent. Once incorporated into an amorphous region, water can increase the free volume and lead to enhanced molecular mobility of the solid causing a reduction in the glass transition temperature. If the T_g is lowered sufficiently, the amorphous material can crystallize, lowering the surface energy. During recrystallization, water, which was present in the amorphous regions, is expelled from the solid. From Figures 7 and 8 it can be seen that the water loss during conversion occurs at a spe-

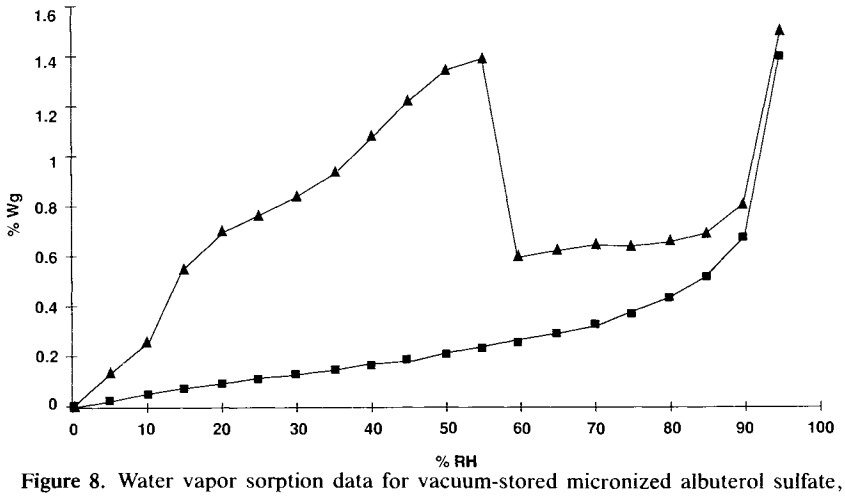

Figure 8. Water vapor sorption data for vacuum-stored micronized albuterol sulfate, sequential scans (▲—first scan, ■—second scan).

cific RH (50%). This crystallization event occurs within 50 minutes, significantly faster than the absorption phase (≈ 200 minutes), indicating that conversion happens only after a threshold quanitity of water is sorbed.

The weight loss observed for micronized albuterol sulfate at 50% RH appears to be irreversible. This effect is demonstrated in Figure 8 where the WVSA scan (absorption only) of the vacuum stored, micronized sample of Figure 7 is compared with the identical material on a sequential WVSA scan. Note that the weight loss at 50% RH does not occur on the second scan. Micronized material stored at 40°C/75% RH also showed no weight loss at 50% RH (or any other relative pressure) and more closely resembled the twice scanned micronized and unmicronized samples. Unmicronized albuterol sulfate stored at high temperature and humidity showed no significant changes in sorption/desorption behavior (data not shown).

It is worth noting here that the USP specification for water content on unmicronized albuterol sulfate is 0.5% w/w. From Figures 7 and 8 it is clear that the moisture levels of this compound are highly dependent upon relative humidity and the processing and exposure histories of the sample. Indeed, the USP moisture specification for unmicronized albuterol sulfate is exceeded for RH values greater than 80% and is especially inappropriate for the freshly micronized powder.

Combined Effects of Micronization and Environmental Exposure

As mentioned previously, particle size reduction techniques such as micronization can induce alterations in the physical state of certain powders. In particular, the input of large quantities of energy during comminution can impart amorphous character to once highly crystalline particles. Figure 1 shows a representation of the first few molecular layers of a crystalline solid. Following micronization these surface layers can become disrupted, leaving an amorphous layer at the particle surface. Often the amorphous regions constitute only a small percentage of the total mass.

Exposure of a partially amorphous solid to water vapor and/or heat can lead to significant changes in the state of the powder. Figure 9 shows one possible scenario involving a moisture mediated amorphous to crystalline conversion. As shown in the figure, if the particles are in contact during crystallization, the result can be interparticle bridging or fusion. Significant agglomeration of micronized particles can, in the worse case, effectively cancel out previous particle size reduction efforts. Scanning electron microscopy of albuterol sulfate exposed to 40°C/75% RH show this to be the case.

CONCLUSION

Micronization processes transfer significant energy to pharmaceutical powders, often producing thermodynamically unstable states. These processes impart a dynamic character to powder systems which can be difficult to predict. Certain environmental conditions can have a significant impact on these powders, inducing further physical changes.

Small differences in crystallinity between micronized and unmicronized powders can be difficult to detect. More importantly, the prediction of crystallinity changes in micronized powders induced by environmental exposure can

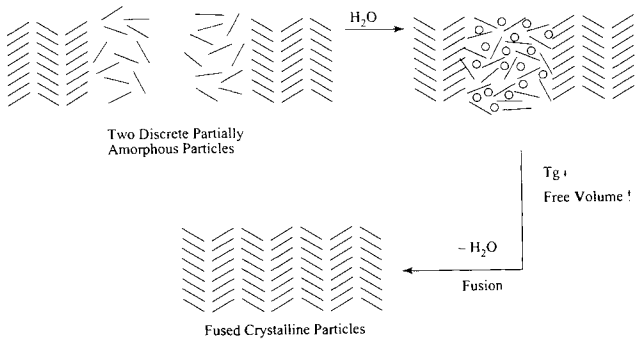

Figure 9. Schematic representation of the crystalline surfaces of two discrete particles after milling. Amorphous regions absorb a sufficient quantify of water (circles) to increase the free volume and lower the T_g. The molecules, now having increased mobility, rearrange into a stable crystalline structure (expelling the water) forming a bridge between the two particles.

be equally difficult. These changes can dramatically reduce the effectiveness of powder inhalation products. An understanding of subtle physical property changes in solids which would allow for the prediction of powder instabilities would be extremely valuable. Only by using a variety of analytical methods can the elucidation of small differences in powder crystallinity be fully ascertained. Scanning electron microscopy was useful in detecting changes in particle morphology resulting from environmental exposure. Laser diffraction also proved to be an effective technique for detecting changes in micronized albuterol sulfate after high temperature and humidity exposure. However, these methods were only able to give information after the fact: no indication of the powders' metastable state was observed.

In addition, for PXRD, no clear indications of differences between the various powder samples were observed under any condition. Differential scanning calorimetry, SMC and WVSA proved to be more predictive of albuterol sulfate physical instability than other methods, with WVSA having the advantage of showing the actual temperature and relative humidity where the conversion occurs. However, once detected, most of the methods explored in this report would be useful in monitoring state changes under different environmental conditions.

The importance of understanding and controlling powder dynamics can not be overstated. In order to predict the reaction of powder and formulation to various environmental stresses, thorough characterization and understanding of the pure powder properties is necessary. Flow characteristics, deagglomeration, and suspendability are some of the ways subtle powder changes can have a dramatic impact on the properties of the final aerosol dosage form. Quantitation of the state of a pharmaceutical powder prior to formulation will result in less raw material induced variation, in effect leading to more robust formulations and processes.

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