The Effect of Bulking Agent on the Solid-State Stability of Freeze-Dried Methylprednisolone Sodium Succinate

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The rate of hydrolysis of methylprednisolone sodium succinate in the freeze dried solid state at 40°C was determined in the presence of two common bulking agents - mannitol and lactose - at two different ratios of drug to excipient. Residual moisture levels were less than 1% in all samples tested, with no significant difference in residual moisture among different formulations. Rate of hydrolysis was significantly higher in mannitol-containing formulations versus lactose-containing formulations, and the rate of hydrolysis increases with increasing ratio of mannitol to drug. Thermal analysis and x-ray diffraction data are consistent with a composition-dependent rate of crystallization of mannitol in the formulation and its subsequent effect on distribution of water in the freeze-dried matrix. Increased water in the microenvironment of the drug decreases the glass transition temperature of the amorphous phase, resulting in an increased rate of reaction. The physical state of lactose remained constant throughout the duration of the study, and the rate of hydrolysis was not significantly different from the control formulation containing no excipient. Thermal analysis and x-ray diffraction data are consistent with formation of a liquid crystal phase in freeze-concentrated solutions of methylprednisolone sodium succinate containing no excipient.

KEY WORDS: thermal analysis; x-ray diffraction; excipients; crystallization; lyophilization.

INTRODUCTION

Bulking agents are commonly used in formulation of freeze dried products in order to provide an inert, easily reconstituted matrix containing a low dose of active drug substance. The study reported here was prompted by the need to prepare material for a blinded clinical trial where three different doses of drug are presented such that the freeze dried cakes all look alike by using an appropriate amount of a suitable bulking agent.

Methylprednisolone sodium succinate is a soluble prodrug of methylprednisolone used as an injectable corticosteroid, where solubilization is achieved through the use of the ionizable hemisuccinate moiety. The prodrug is unstable in aqueous solution, the principal products being hydrolysis to methylprednisolone and acyl migration to form the isomeric 17-ester (1). Therefore, methylprednisolone sodium succinate is marketed as a freeze dried powder (Solu-Medrol®).

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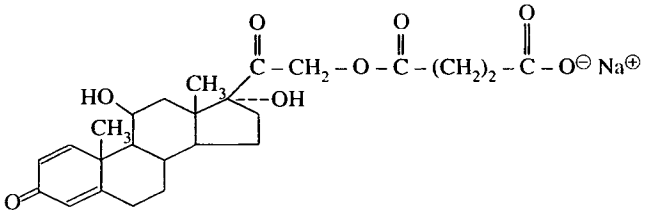
The purpose of this study is to examine the effects of two of the most commonly used bulking agents in freeze dried injectable formulations- mannitol and lactose - on the stability of methylprednisolone sodium succinate as a freeze dried solid, with particular attention to the effect of excipient on the physical form of the freeze dried solid and the location of water within the solid matrix.



MATERIALS AND METHODS

Materials

Methylprednisolone hemisuccinate USP was provided by The Upjohn Company, Kalamazoo, MI. Mannitol and lactose were USP grade and were either provided by The Upjohn Company or purchased from Sigma Chemical Co. Phosphate buffer salts (Fisher Scientific) were analytical grade. Inorganic salts for water vapor adsorption experiments were reagent grade materials.

Methods

Drug with no excipient present (250 mg per vial) was used as a control for stability studies. Two strengths of drug were examined with each bulking agent - 40 mg and 125 mg - where the total amount of bulking agent plus drug was the same as the control. The formulation was prepared by suspending methylprednisolone 21-hemisuccinate in 0.08 M phosphate buffer, pH 7.5, containing the appropriate quantity of either mannitol or lactose. Conversion to the sodium salt was carried out by slow addition of 10% sodium hydroxide until essentially all solids were dissolved. The final pH was 7.5 - 7.7. Solutions were sterile filtered and 3.74 ml was filled into 20 ml tubing glass vials. Freeze drying was carried out by placing vials directly on the freeze dryer shelf, freezing at -50°C for 4-6 hrs, followed by primary drying at a shelf temperature of 10°C and a chamber pressure of 100 microns Hg for 24 hrs. Secondary drying was carried out for approximately 24 hrs at a shelf temperature of 30°C and a chamber pressure of 100 microns Hg. Vials were stoppered under full vacuum. Residual moisture was measured by Karl Fisher titration (Model 701, Metrohm, Inc., Herisau, Switzerland) where the freeze dried material was quickly transferred to the titration vessel containing anhydrous methanol. Coulometric end point detection was used.

Stability of the freeze dried solid was measured at 25°C and 40°C using a reverse phase HPLC assay (1). A 4 μm Nova-Pak® C-18 column (Waters, Inc., Milford, MA) was used. The mobile phase consisted of 33% acetonitrile in water buffered at pH 5.2 - 5.4 with 0.05M acetate buffer. A fixed wavelength UV detector at 254 nm and digital data station were used to quantitate the parent compound and degrada-

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tion products. USP reference standards of methylprednisolone hemisuccinate and methylprednisolone were used as external standards. Percent methylprednisolone, the ester hyrolysis product, was used as the primary measure of chemical stability in the solid state.

Both the freeze dried solids and solutions prior to freeze drying were examined by thermal analysis using a Perkin-Elmer Series 7 DSC with a mechanical cooling accessory and computer data station (2). Helium (40 ml/min) was used as the purge gas. Solution samples were prepared by placing approximately 20 µl of solution in an aluminum sample pan and sealing the sample by crimping an aluminum cover in place. Samples were frozen at a controlled rate to about -50°C, and the thermograms were recorded during warming at a controlled rate to just above 0°C. A nitrogen-purged glove box was placed over the sample compartment to prevent artifacts due to moisture condensation. Solid samples were prepared by packing about 10 mg of freeze dried powder into aluminum sample pans. For measuring the effect of water vapor sorption on glass transition temperatures in the solid state, DSC pans containing the solid sample were placed in dessicators at different relative humidities and equilibrated for about 48 hours, at which time the samples were quickly removed from the dessicator and sealed. Thermograms were recorded in the range of 20-120°C.

Water vapor sorption isotherms were measured on freeze dried powders by placing vials (with lyostoppers in the partially inserted position) in dessicators containing various saturated salt solutions. The following salts were used - sodium hydroxide, lithium chloride, potassium acetate, potassium carbonate, and sodium bromide. Samples were removed at two hour intervals during the first 12 hours, followed by sampling twice a day for five days. Vials were sealed upon removal from the dessicator, and moisture content was determined by Karl Fisher titration. The water content of the samples was found to reach approximate equilibrium after about 48 hours at room temperature.

The physical form of the freeze dried powders was studied by x-ray powder diffraction. A Siemens Krystalloflex® diffractometer was used, with CuK_α radiation at a voltage of 40 kV and a current of 20 mA. Powder specimens were prepared by gently breaking up the freeze dried cakes and placing in an aluminum powder mount. Samples were scanned from 2° to 40° at 0.1° per second.

RESULTS AND DISCUSSION

Effect of Bulking Agent on Stability

The stability at 40°C of the control formulation is shown in Figure 1 along with that of formulations containing 40 and 125 mg of drug in mannitol and 40 mg of drug in lactose. Each data point represents the average of duplicate measurements from different vials. Rate of hydrolysis of the drug with mannitol as the bulking agent is markedly faster than when lactose is used, and the rate of hydrolysis increases as the ratio of mannitol to drug increases. The rate of hydrolysis of the 40 mg strength in lactose is not significantly different from that of the control.

Residual moisture content of all formulations were in the range of 0.3 to 0.9% percent, and there was no significant difference in residual moisture between the samples containing mannitol and those containing lactose. There was a small but significant increase in residual moisture of all formulations during six months storage at 40°C, probably caused by water vapor transfer from the rubber stopper to the freeze dried cake. The average increase was 0.29% with a range of 0.05 - 0.45% and there was no significant difference between the formulations studied with respect to increase in residual moisture level.

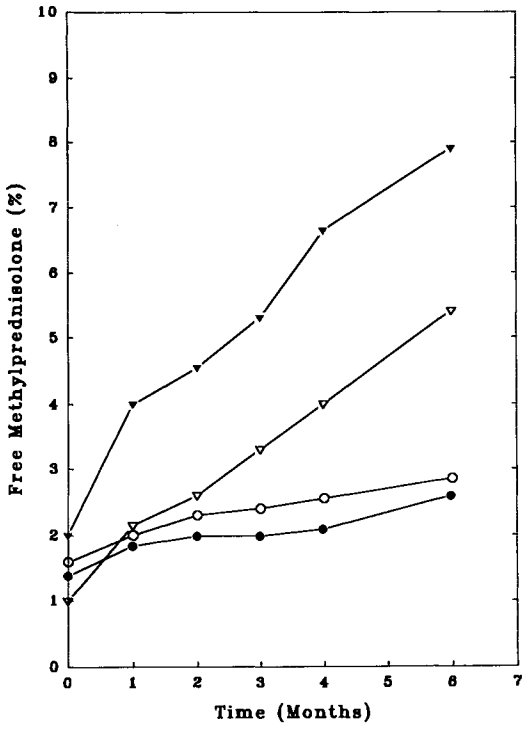

Figure 1 - Hydrolysis of methylprednisolone sodium succinate at 40°C for 250 mg control (solid circles), 125 mg drug in mannitol (open triangles), 40 mg drug in mannitol (closed triangles), and 40 mg drug in lactose (open circles).

Thermal Analysis

DSC thermograms of the formulations prior to freeze drying are shown in Figures 2-4. Note that endotherms are represented by upward deflections on all thermograms. Thermograms of methylprednisolone sodium succinate in phosphate buffer with no bulking agent present (Figure 2) are shown for three different heating rates after cooling at a rate of 20°C/min. Three separate thermal events occur prior to the melting endotherm of ice, and the thermograms recorded at different heating rates illustrate the effect of heating rate on both sensitivity and resolution. The endotherm on the leading edge of the ice melting endotherm is resolved at the slowest heating rate, but is not resolved at the highest heating rate. Two exotherms are observed prior to the endotherms, with best sensitivity in detecting the exotherms observed at higher heating rates.

The thermogram in Figure 2 is not consistent with low-temperature thermal behavior of solutions in which the solute a) crystallizes readily upon freezing, b) remains amorphous upon freezing, or c) initially forms a metastable amor-

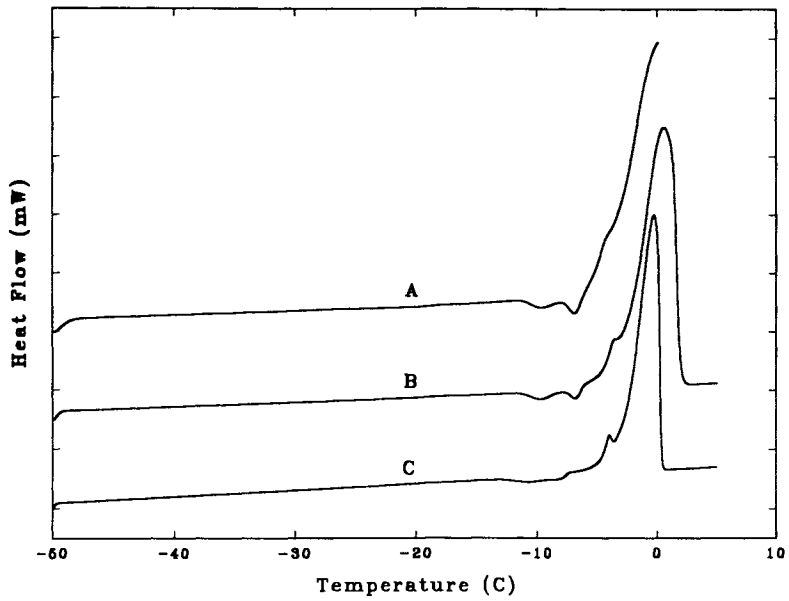

Figure 2 - DSC thermograms of methylprednisolone sodium succinate in 0.08 M phosphate buffer. Cooling rate was 20°C/min and heating rates were at 10°C (A), 5°C (B), and 2°C (C) per minute.

phous phase which then crystallizes with subsequent warming (3,4). No glass transition is observed in Figure 2, as would be expected for an amorphous solute. The freeze dried drug does undergo a glass transition, however (see Figure 5). The endotherm on the leading edge of the ice melting endotherm suggests eutectic melting; however, the x-ray diffractogram of the freeze dried drug alone (see Figure 7) is not consistent with well-defined crystallinity.

Thermograms of the lactose formulations (Figure 3) show a glass transition (T_g') with a midpoint of about -27°C. This is consistent with the behavior of a solution where the solute does not crystallize when the system is frozen. The relative concentrations of lactose and drug have no significant effect on T_g' for the formulations studied here. This result is in reasonable agreement with a previously reported value of about -29°C for T_g' of lactose (2).

Thermograms of the mannitol formulations (Figure 4) indicate that the physical state of the material depends on the ratio of drug to mannitol. For the formulation containing 40 mg of drug, a crystallization exotherm is observed in the temperature range expected for mannitol. The formulation containing 125 mg of drug in mannitol does not crystallize under the conditions used for thermal analysis. Instead, a glass transition is observed, with a mid-point temperature of about -36°C. This is lower than the glass transition temperature of mannitol alone (about -28°C). This may be explained

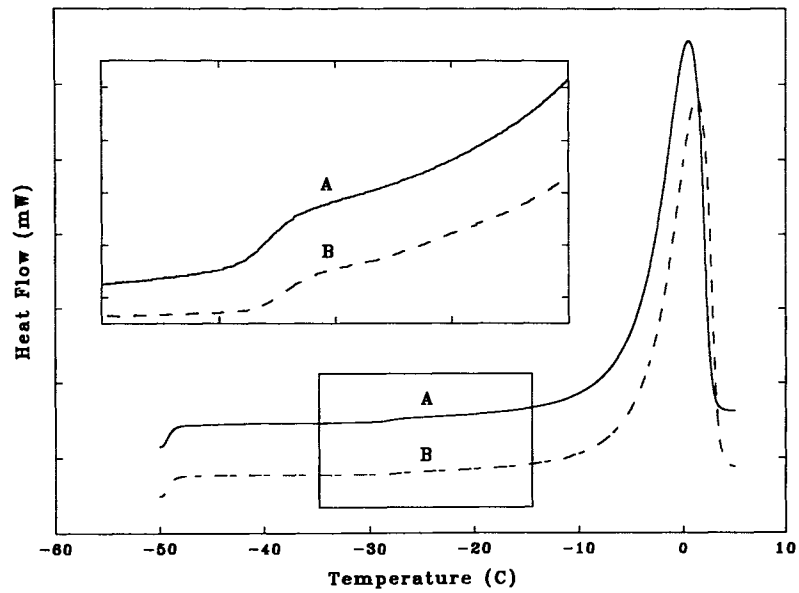

Figure 3 - DSC thermograms of formulations containing 40 mg (A) and 125 mg (B) of drug in lactose. Inset shows glass transition region.

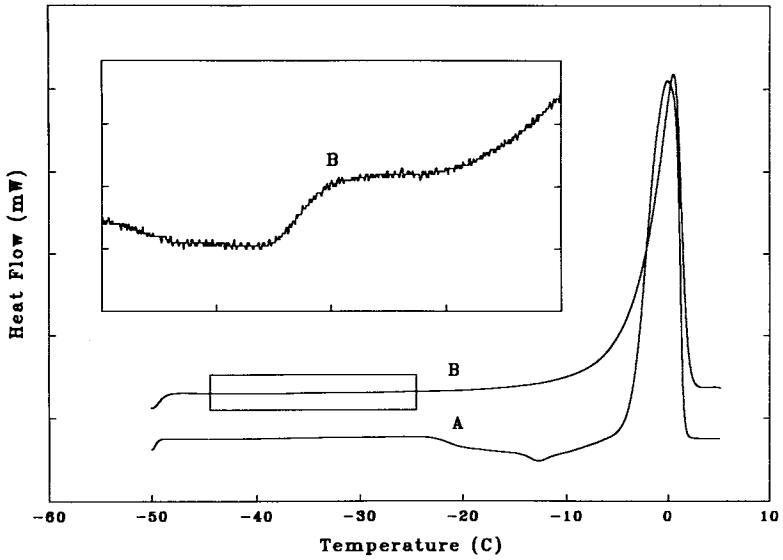

Figure 4 - DSC thermograms of formulations containing 40 mg (A) and 125 mg (B) of drug in mannitol.

by a plasticizing effect of the drug itself or, more likely, increased unfrozen water in the freeze concentrate caused by the drug, the buffer, or both.

Thermal analysis of freeze dried drug (no excipient present) shows a glass transition which is well above 100°C at low residual moisture levels. As shown in Figure 5, the glass transition temperature decreases continuously as the residual moisture level increases. This is consistent with the behavior of amorphous solids where water acts as a plasticizer, as previously reported by Zografi and coworkers (5,6).

X-Ray Powder Diffraction

X-ray powder diffractograms of a mannitol-containing formulation (125 mg drug) are shown in Figure 6 initially and at 2 and 6 months. The data show that the freeze dried solid is substantially amorphous initially, and that crystallinity develops during storage despite residual water levels on the order of 1.0 to 1.5 percent. The formulations containing lactose as the excipient were amorphous initially, and remained so for the duration of this study (data not shown). The formulation containing 40 mg of drug in mannitol showed substantial crystallinity initially after freeze drying, and the degree of crystallinity increased somewhat during the six month storage interval (data not shown). Samples of freeze dried mannitol alone were prepared for use as a standard to estimate the degree of crystallinity in stability samples; however, both mannitol alone and mannitol in the formulation appear to be mixtures of polymorphs (7), and the composition of the mixtures is not the same for the two materials. Further work is under way to better characterize the crystal forms of mannitol which are formed during freezing and drying under a variety of conditions.

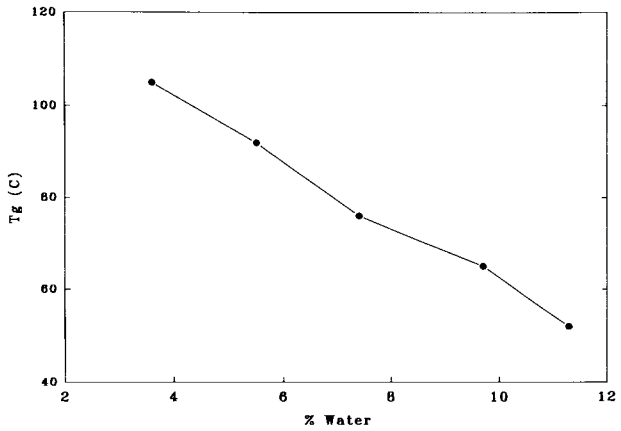

Figure 5 - Glass transition of freeze dried methylprednisolone sodium succinate as a function of water content.

The x-ray diffraction pattern of drug with no bulking agent present is shown in Figure 7. A single, sharp peak was observed in all vials tested at about 4° 2θ, and a broad, diffuse peak is centered at around 18° 2θ. This type of diffractogram is consistent with diffractograms reported for liquid crystals (8), where the reflection at the low angle arises from the spacing between layers, and the larger angle reflection arises from order within the individual layers. Additional polarized light microscopy experiments have confirmed the formation of liquid crystals by methylprednisolone sodium succinate. A lyotropic mesomorphic phase might also explain the endotherm observed in Figure 2 prior to the main ice melting endotherm. Casillas and coworkers (9) observed two endotherms prior to the main ice melting endotherm in the thermogram of aqueous solutions of dioctyl sodium sulfosuccinate (Aerosol OT). These additional endotherms were attributed to water in two different states of association with the solute in the mesophase.

Given that the surface activity of the drug is well documented (10), formation of a liquid crystalline phase from concentration of a micellar system would not be unexpected. However, further work is needed in order to better understand the pharmaceutical significance of the liquid crystalline state within freeze dried solids. While the solid state stability of methylprednisolone sodium succinate in the absence of any excipient is not a problem at sufficiently low water content, the general significance of liquid crystal phase formation with respect to stability could be related to

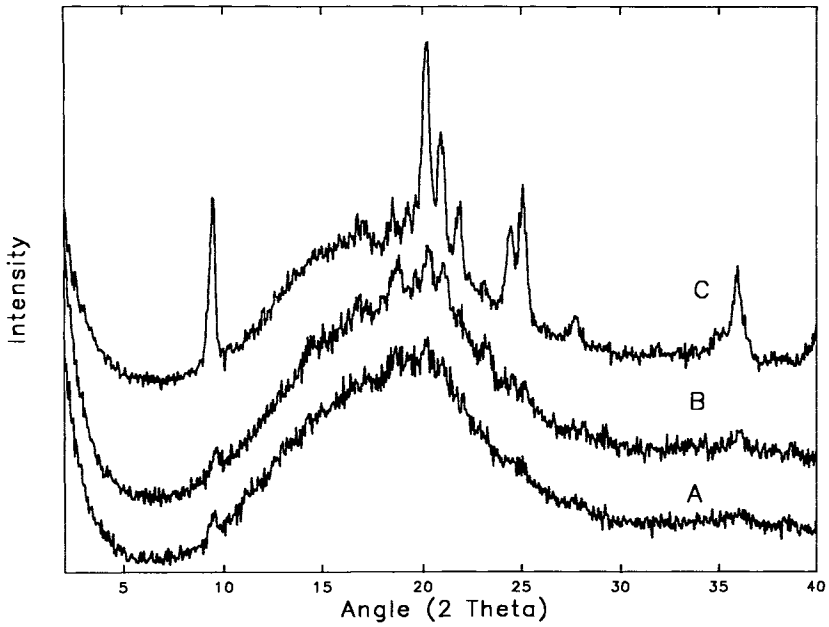

Figure 6 - X-ray powder diffractograms of formulations containing 125 mg of drug in mannitol initially after freeze drying (A), and after 2 months (B) and 6 months (C) storage at 40°C.

preferred orientation between molecules in the liquid crystal phase, with subsequent effects on reaction rates through intermolecular catalysis. As a side note, it was observed during x-ray diffraction analysis of stability samples that some vials of drug containing no excipient show evidence of crystallization during storage. Further work is needed to better understand the phase transitions involved; in particular, whether a crystalline solid is formed from the dehydrated liquid crystal phase, or whether the liquid crystalline phase must first convert to an amorphous phase prior to crystallization of the solid.

Water Vapor Sorption Isotherms

Water vapor adsorption isotherms were measured for freeze dried, single component systems consisting of mannitol, lactose, and methylprednisolone sodium succinate in order to compare water uptake characteristics of the individual formulation components. Moisture content versus time at relative humidities of 11, 21, 32, and 43 percent was determined. The approximate time for a reasonably constant moisture level to be established was 48 hours at all relative humidities examined. The plateau value of moisture was

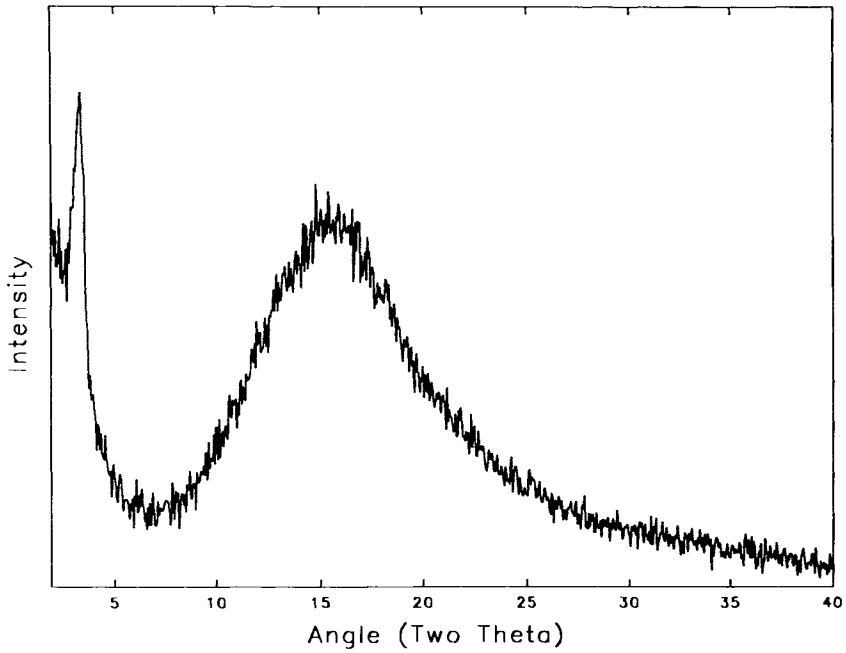

Figure 7 - X-ray diffraction of freeze-dried methylprednisolone sodium succinate with no excipient present.

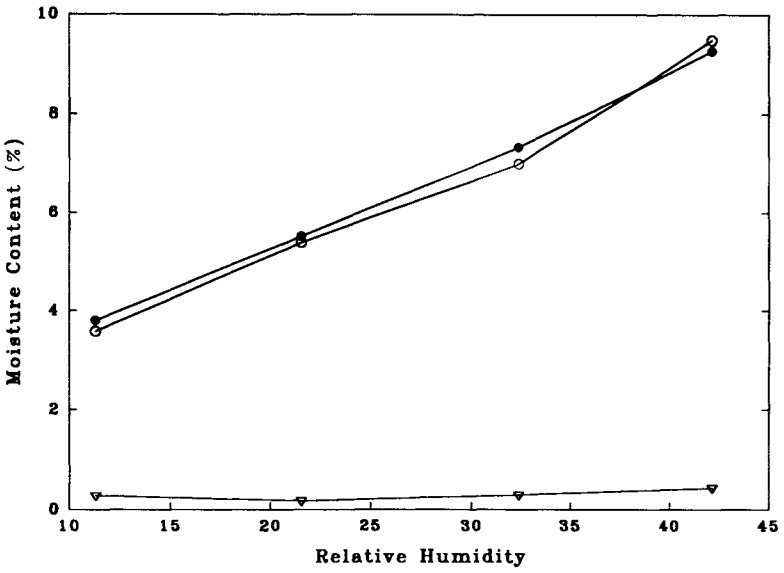

Figure 8 - Water vapor adsorption isotherms of freeze dried mannitol (open triangles), lactose (open circles), and drug (closed circles).

plotted against relative humidity to construct the water vapor sorption isotherms shown in Figure 8. Lactose (amorphous) and methylprednisolone sodium succinate (dried liquid crystal) are similar in water vapor sorption characteristics. Mannitol, however, adsorbs very little moisture for the relative humidity range examined. This is consistent with crystalline material, where the only surface available for water vapor sorption is the surface of crystals.

Suggested Role of Excipient in Solid State Stability

The data reported here are consistent with the work of Zografi and coworkers which support the idea that the effect of water on critical attributes of amorphous drugs is determined not so much by how much water is present, but by where the water is located. The effect on critical product attributes of concentration of water within amorphous regions becomes more important as the fraction of amorphous material decreases (11). In formulations containing mannitol as the excipient, the mannitol crystallizes either during the freeze dry process or with time during storage, depending on the ratio of mannitol to drug. In either case, there is not a significant amount of water associated with crystalline mannitol, as shown by the comparative water vapor sorption isotherms. For the formulation containing 40 mg of drug in mannitol, the effective water content of the amorphous phase would be expected to be more than five times the value measured by Karl Fisher titration, assuming that the mannitol is essentially all crystalline, since the mannitol to drug ratio is approximately 5.2:1. Referring to Figure 5, the increased water level would be expected to decrease T_g' by 20° to 30°C. Amplification of the water content of the amorphous phase could occur by any or all of three mechanisms: 1) While the initial levels of residual moisture are not significantly different for lactose and mannitol formulations, the amount of water "seen" by the drug cannot be the same given the dramatically different water vapor adsorption isotherms for the two materials. 2) Crystallization of mannitol with time would result in a redistribution of water in the freeze dried matrix and an increase in the amount of water in the drug microenvironment. 3) Water added to the freeze dried solid by transfer from the stopper would locate in the amorphous phase. Subsequent lowering of the glass transition temperature of the amorphous phase would be expected to accelerate the rates of chemical reactions leading to instability of the drug substance.

In contrast to the mannitol formulations, those containing lactose are markedly more stable because lactose remains amorphous, resulting in a more uniform distribution of water in the freeze dried matrix. This is supported by the water vapor adsorption isotherm data, showing similar affinity of both drug and lactose for water. The similar water vapor adsorption isotherms support the idea that lactose could act, in part, as an internal dessicant by competing for the available water.

Practical Implications

Mannitol is often the first choice as a bulking agent among formulation development scientists, since it is inexpensive, non-toxic, and can be freeze dried at relatively high temperatures with full retention of the desireable properties of a freeze dried product. Lactose, on the other hand, requires more conservative drying conditions because it remains amorphous on freezing and has a relatively low T_g' temperature of about -30°C. However, it is important to be aware of potential adverse effects on stability because of the effect of mannitol crystallization on distribution of water in the freeze dried cake. Stability protocols for mannitol-based formulations should reflect the potential for adverse effects of mannitol on stability of drugs which are amorphous as freeze-dried solids and, in particular, for protein formulations containing mannitol (12). Particular attention should be given to monitoring moisture content of stability samples in mannitol-based formulations, since water vapor transfer from the stopper to the product can be very significant, particularly when the moisture is concentrated in amorphous regions of the lyophilized cake.

Looking at only the DSC thermogram of the drug solution at low temperature, a development scientist would be led to believe that the drug crystallizes during freezing or after the frozen solution is heated during initiation of drying. This drug is certainly not crystalline in the traditional sense after freeze drying. This points to the importance of characterizing the dried solid by x-ray diffraction initially after freeze drying and monitoring the physical state of the solid during stability studies.

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