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RESEARCH PAPER

Physicochemical Stability of Crystalline Sugars and Their Spray-Dried Forms: Dependence upon Relative Humidity and Suitability for Use in Powder Inhalers

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ABSTRACT

Lactose, trehalose, sucrose, and mannitol were purchased in crystalline form and fractionated by sieving. Coarse (125–212 μm) and fine (44–74 μm) free-flowing fractions were selected as typical of drug carriers in dry-powder inhalers. In addition, one batch of each sugar was spray-dried to form a respirable powder (> 50% [w/w], < 5 μm). Both fractions and the spray-dried powders were characterized before and after storage for 30 days at < 23%, 23%, 52%, 75% and 93% relative humidity (RH) at 25°C. Moisture uptake was determined by thermogravimetric analysis (TGA) validated by Karl Fischer titration. Sieve fractions (before storage at different RHs) and spray-dried materials (before and after storage) were further characterized by differential scanning calorimetry (DSC) and x-ray powder diffraction (XRPD). All crystalline sieve fractions (except sucrose at 93% RH) were stable at 25°C and showed insignificant moisture uptake when exposed to each relative humidity for 30 days. Sucrose dissolved in sorbed moisture at 93% RH. Spray-dried lactose, sucrose, and trehalose, which were collected in the amorphous form, showed moisture uptake, without recrystallization, when held for 30 days at 23% RH. These sugars recrystallized as sintered masses and became undispersible at ≥ 52% RH. Spray-dried mannitol was apparently 100% crystalline when collected directly from the spray-dryer; it did not show humidity-induced changes.

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The physicochemical behavior of each sugar form is discussed as it relates to the sugar's suitability as a powder-inhaler excipient, with both conventional and protein drugs.

INTRODUCTION

Propellant-free, powder inhalers are being developed to avoid the problems associated with the replacement of chlocoflurocarbons (CFCs) in pressurized metered-dose aerosols (1). They also show promise for the delivery of aerosolized peptides and proteins to the lung, prior to these compounds' systemic absorption (2,3). For both conventional drugs and macromolecules, the active ingredient is usually diluted with lactose (4). From a toxicological point of view, however, most sugars are likely to be acceptable for this purpose. Powder formulations for conventional low-molecular-weight drugs usually involve the preparation of an ordered mixture of a micronized (jet-milled to < 5 μm, and respirable) crystalline drug with a relatively free-flowing (larger particle sizes: > 40 μm) crystalline sugar, such as α-lactose monohydrate. The flow characteristics of the sugar aid in packaging and emptying of the dosage form during use (4). Ideally, drug particles should be attached to the surface of the larger carrier particles with forces of sufficient magnitude to prevent de-segregation (during dosage-form manufacture and storage). Conversely, these interparticulate adhesion forces must be small enough to enable drug detachment from the carrier in the turbulent air-stream created by the patient's inhalation (5). Because different drugs have different adhesion properties, and these properties depend also on the excipient chosen as carrier, a larger excipient menu would clearly be useful.

Powder formulations for proteins may require excipient dilution for different reasons. Biological macromolecules can rarely be milled in conventional ways (6), and although spray drying may be used to create respirable particles (majority < 5 μm) (7,8), proteins may denature during the process. Thus, sugar diluents have been used to stabilize proteins, both during drying and storage in the solid state (9–13). However, sugar selection and the concentration dependence of stabilization remain subjects of discussion and review (14,15).

Unlike the situation in which a mixture of drug and large carrier particles is used to deliver mostly micronized drug to the lung (carrier particles are not intended for inhalation), spray-dried sugars in this latter context must form solid-state molecular mixtures with the protein or peptide drug in question. These protein–sugar mixtures must be prepared as powders in respirable particle sizes, prior to dispersion and inhalation of both sugar and drug.

Consequently, in addition to their offering protection to labile proteins, there is an opportunity to use sugar diluents to create more predictable and dispersible powder products for use in inhalers, when these sugars are spray-dried and collected as respirable powders. However, because spray-drying often leads to the formation of metastable, high-energy amorphous forms (16,17), which may recrystallize over time and render a formulation "nonrespirable" (18), some sugars may be more suitable than others as protein and peptide diluents in powder inhalers. Ideally, formulations should be unaffected by storage or use under different environmental conditions (19).

This article reviews the suitability of four different sugars for use as excipients in dry-powder inhalers. Three of the sugars, lactose, sucrose, and mannitol, are widely available in USP-NF pharmaceutical grades. A fourth sugar, trehalose, is currently being considered as a new excipient, because of its reported protein stabilizing properties (20). All four sugars were assumed to be toxicologically acceptable for inhalation. Each sugar was characterized in the form of spray-dried, respirable particulates, and as two, typical crystalline sieve fractions, before and after 30 days of storage at different relative humidities. Their physicochemical behavior is discussed as it relates to the suitability of each form of sugar as a powder-inhaler excipient, as well as the need to protect formulations from humidity-induced changes in powder characteristics.

MATERIALS AND METHODS

Materials

D-Mannitol, sucrose, and α,α-trehalose dihydrate were obtained from Sigma Chemical (St. Louis, MO). α-Lactose monohydrate was obtained from Foremost Ingredient Group (Baraboo, WI). Karl Fischer reagents, Aquamicron-AS,® and Aquamicron-CS,® were obtained from Cosa Instrument Company (Norwood, NJ). All other chemicals were obtained from Fisher Scientific (Raleigh, NC) and were reagent grade.

Table 1

Experimental Conditions for Spray-Drying (Yamato ADL-31) of Sugars from 10% (w/v) Aqueous Solutions

Condition	Lactose	Trehalose	Sucrose	Mannitol
Inlet temperature (°C)[a]	150 (147–154)	125[b] (122–132)	125[b] (118–125)	150 (142–154)
Outlet temperature (°C)a	70 (73–76)	70 (59–64)	70 (60–63)	70 (73–77)
Atomizing pressure (kgf/cm^2)[a]	3.0 (2.8–3.0)	3.0 (2.8–3.0)	2.8 (2.8–3.0)	3.0 (2.8–3.0)
Feed flow rate (mL/min)	10	10	10	10
Aspirator setting	4	2[c]	4	4

[a] Values are instrument settings (recorded values).
[b] 125°C was used, since a crusty product was obtained when 150°C was employed.
[c] Aspirator setting was held at 2 to prevent powder loss from the collection vessel into the air-suction hose.

Sieve Fractionation of Sugars

Sugars were sieved in a sieve shaker (Cenco-Meinzer, Central Scientific, Chicago, IL). Sucrose crystals were lightly crushed in a mortar to comminute and remove agglomerates prior to sieving; other sugars were sieved as received. Sieve fractions that were thought representative of those used in inhalation products were retained. These are designated "coarse fraction" A (125–212 μm), and "fine fraction" B (44–74 μm) throughout this article. After fractionation, A and B were retained in tightly closed amber bottles and stored in desiccators over phosphorus pentoxide prior to further study.

Spray-Drying of Sugars

A 10% (w/v) aqueous solution of each sugar was spray dried using a Yamato ADL-31 Mini-Spray Dryer (Yamato Scientific America, Orangeburg, NY). Inlet temperatures, atomizing pressure, and other spray-drying conditions are summarized in Table 1. Spray-dried sugars are designated as C throughout this article. Immediately after their collection, sugars were packed into tightly closed amber bottles and stored in desiccators, over phosphorus pentoxide, prior to study.

Moisture Sorption Studies

Approximately 2 g samples of each sugar in forms A, B, and C were spread uniformly in open Petri dishes (Cat. No. 09-753-52A, Fisher Scientific, Raleigh, NC). Each sample was immediately transferred to a designated "desiccator vessel" (Cat. No. 08-595-2E, Fisher), with a saturated salt solution in its base, for timed exposure to a fixed-relative-humidity (RH) environment (21). Salts used were potassium acetate (23% RH), magnesium chloride (52% RH), sodium chloride (75% RH), and potassium nitrate (93% RH). The desiccator vessels were housed inside an environmental cabinet (Model 435314, Hotpack, Philadelphia, PA) maintained at 25°C. Samples were reanalyzed after 30 days of storage.

Differential Scanning Calorimetry

Samples of the crystalline coarse and fine sieve fractions (A and B) and the spray-dried sugars (C; before and after 30 days of storage at 25°C and different RH) were studied using a differential scanning calorimeter (DSC 7, Perkin Elmer, Norwalk, CT). Samples of 2–10 mg, accurately weighed in crimp-sealed (non-hermetic) aluminum sample pans, were scanned from 25–250°C at a heating rate of 10°C/min under a nitrogen gas purge. An empty crimped pan served as the reference, and all scans were performed in triplicate. The instrument was calibrated prior to sample analysis, using an indium standard (Perkin Elmer; melting point = 156.6°C, ΔH_m = 28.45 J/g).

Thermogravimetric Analysis

Thermogravimetric analysis (TGA) was performed on sugar forms A, B, and C. Samples were weighed into open pans and analyzed (TGS2, Perkin Elmer, Norwalk, CT). A heating rate of 10°C/min was used at all times, with a nitrogen purge. All scans were performed in triplicate. The instrument calibration check was done with alumel (magnetic transition temperature = 163°C, Perkin-Elmer). After confirmation by Karl Fischer titration (below), the percentage of the initial weight lost during the heating process to 160°C (sucrose), 180°C (trehalose), and 200°C (mannitol and lactose) was ascribed to the water content of each sugar. The temperatures were

chosen as the midpoints of plateau regions in the weight-vs.-temperature profiles.

Particle-Size Determination

Particle-size distributions of spray-dried sugars were determined with an Aerosizer™ equipped with an Aero-Disperser™ (Amherst Process Instruments, Hadley, MA), as described previously by Hindle and Byron (21). Instrument settings were held constant at "shear force" = 0.5 psi, "size limit" = 220 μm, and "feed rate" = 1000 particle counts/sec, with "pin vibration" activated and "deagglomeration" set to high. Size distributions were displayed and calculated, using API Aerosizer MACH2 software V6.02.32, in terms of aerodynamic diameter. The values for true density (employed in the calculation of aerodynamic size distributions) were determined by helium pycnometry on the spray-dried materials (Micromeritics, Norcross, GA), using an outgassing temperature of 27.7°C.

Hot-Stage Microscopy

Hot-stage microscopy (Mettler FP82, Hightstown, NJ) was performed as a diagnostic test on selected samples to better interpret thermal events in differential scanning calorimetry. A scanning rate of 10°C/min with nitrogen purge was used. The hot-stage furnace was cross calibrated with the DSC 7 instrument under these conditions, using stearic acid and indium melting transitions (69.1°C and 156.6°C, respectively). Sample behavior was observed under cross polarized filters, using incident and transmitted light (Nikon Optiphot, Tokyo, Japan).

X-ray Powder Diffraction

X-ray powder diffraction (XRPD) was performed on both sieved fractions of each sugar (A and B) prior to humidity testing. Spray-dried sugars (C) were tested before and after 30 days exposure to different RH environments. Each powder sample was loaded into the sample holder of a Rigaku Geigerflex, Model 2028 x-ray diffractometer (Rigaku Denki, Tokyo, Japan) and depressed with a glass slide to produce a sample bed flush with the surface of the holder. Sugars that had recrystallized to form a fused mass at higher humidities were crushed to powder in a glass mortar prior to sample preparation. All samples were scanned between $2\Theta = 5–45°$, using a Cu target at 35 kV and 22.5 mA. In preliminary experiments, angular positions of the most intense peaks for several of the sugars were compared to Joint Commission on Powder Diffraction Standards (JCPDS) data files (22) to ensure accurate angular calibration of the instrument.

Karl Fischer Moisture Determination

Karl Fischer analysis was performed on the fine-sieve fraction (B) of each sugar, prior to humidity testing, to confirm that the initial weight loss recorded by TGA was due to dehydration. This analysis employed a Mitsubishi Moisture Meter, Model CA-05 (Mitsubishi Kasei, Tokyo, Japan). Between 100 and 300 mg of each sugar were weighed accurately and dissolved in 10 ml of formamide; 400 μl of this solution was injected and titrated coulometrically for water. A blank formamide injection was titrated prior to each sample injection. The water concentration of the blank was subtracted from the water content of the sample. Reported values are the means of five such (sample − blank) determinations.

RESULTS AND DISCUSSION

Much of the discussion of the physicochemical stability of the various forms of the sugars examined in the study will rest on the structures shown in Figure 1 and the data shown graphically in Figs. 2–5. The x-ray powder diffraction patterns (Figs. 2 and 3) and thermograms (Figs. 4 and 5) are broad summaries that enable a discussion of the major physical transformations seen in these investigations. Tables 2–7, on the other hand, are condensed forms of the data collected during the many experiments performed in the study. They summarize for lactose, trehalose, sucrose, and mannitol the precision of the DSC and TGA determinations, and define experimentally determined ranges of values for water content, dehydration, recrystallization and melting temperatures, and enthalpies as functions of sample-pretreatment and process variables. The data for water content for the various forms of each sugar, before and after storage at different relative humidities (Table 2), are based on thermogravimetric analysis for percent weight loss to the midpoint of each sugar's TGA plateau region (e.g., upper curve of the left frame for trehalose in Fig. 5). These values were more precise than, and correlated well with, Karl Fischer titrations for water, as shown in the footnote to Table 2.

Crystalline Sieve Fractions A and B

All sugars were highly crystalline following sieve fractionation (Figs. 2 and 3, XRPD), but differed with respect to the obtainable yields of the coarse and fine

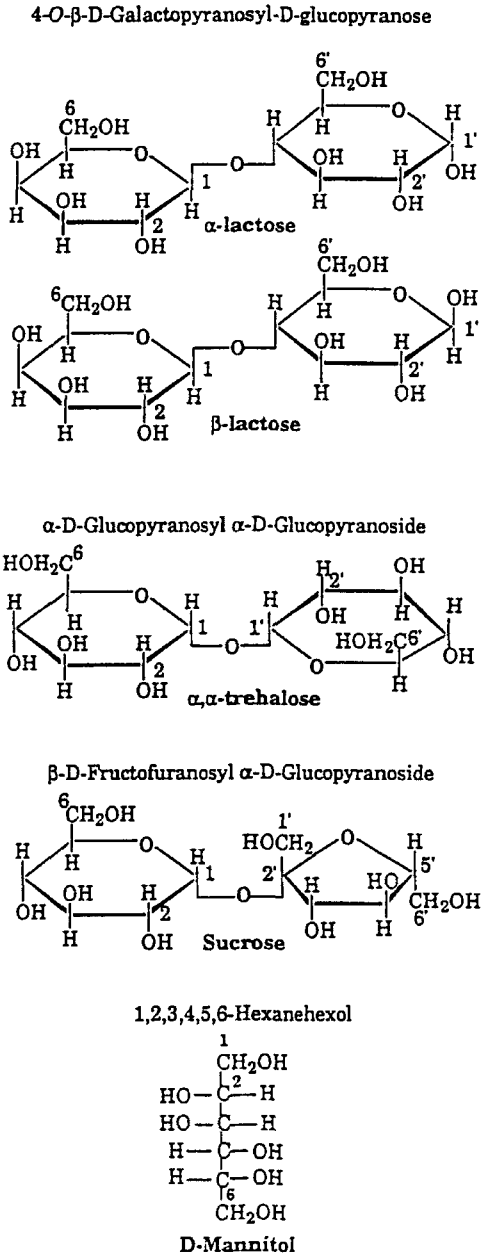

Figure 1. Molecular structures of sugars used in the study. Ring structures are numbered with and without primes (′) only for convenient textual reference. The structure α-lactose is shown, because a mixture of α and β isomers forms quite rapidly in solution, following dissolution of α-lactose monohydrate in water (crystalline β-lactose is anhydrous). The crystalline sugars sucrose and mannitol are anhydrous, while α,α-trehalose is a stoichiometric dihydrate.

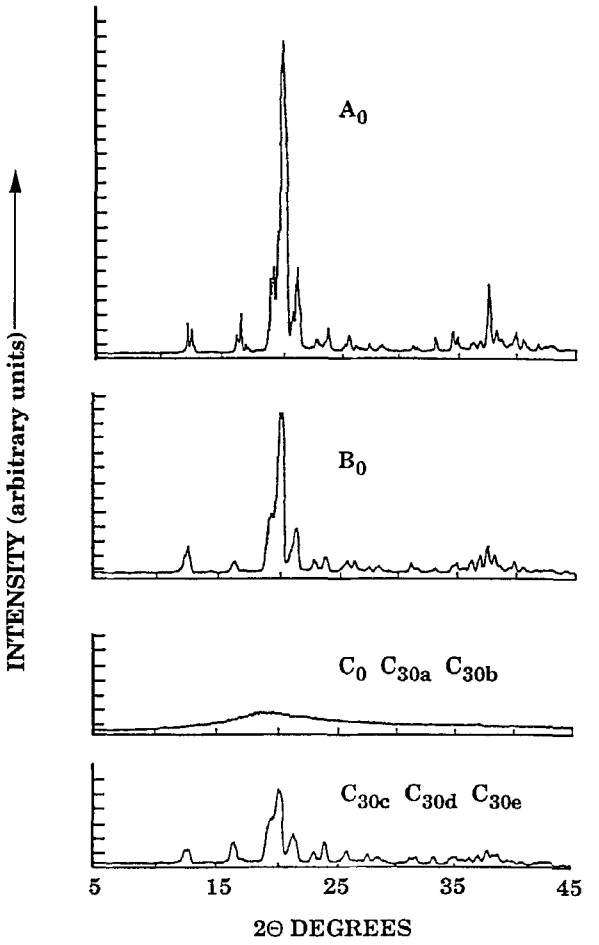

Figure 2. Typical x-ray diffractograms of lactose before (subscript 0) and after (subscript 30) storage for 30 days at 25°C at different relative humidities. A = coarse sieve fraction, B = fine sieve fraction, C = spray-dried. Subscripts: a = stored in sealed bottles over P_2O_5; b–e were stored open at b = 23%, c = 52%, d = 75%, and e = 93% RH. XRPD was not performed on coarse or fine sieve fractions after storage at different relative humidities.

fractions A and B. While lactose is commercially available in these different particle-size distributions (21), yields of A and B were 12% and 3% (trehalose), 3% and 1.8% (sucrose), and 1.6% and 30% (mannitol), respectively. The water-sorption behavior of the stoichiometric hydrates lactose and trehalose (Fig. 1) differed from that of the anhydrous sucrose and mannitol (Table 2). With the exception of sucrose stored at 93% RH, sieve fractions of anhydrous sucrose and mannitol showed undetectable water sorption before or after storage at different humidities. Crystalline sucrose, stored at 93% RH for 30

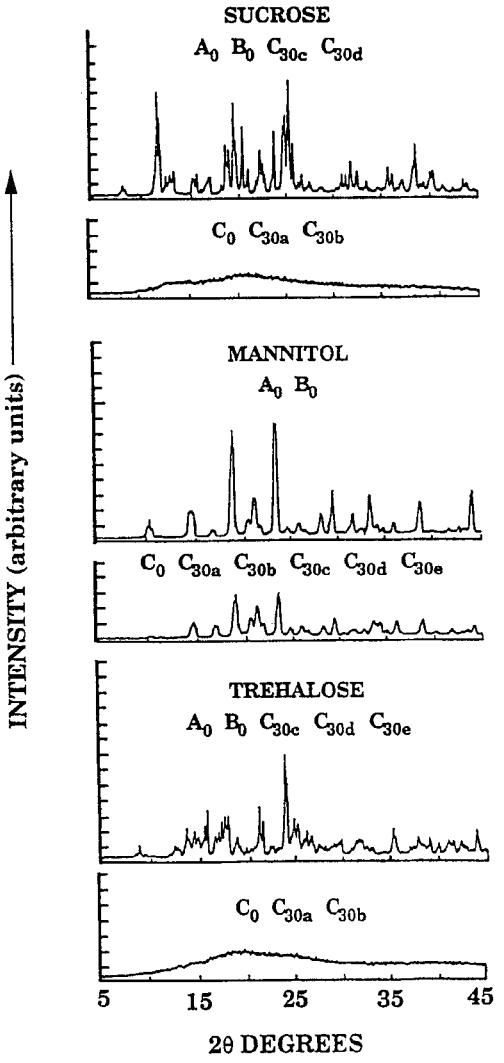

Figure 3. Typical x-ray diffractograms of trehalose, sucrose, and mannitol before (subscript 0) and after (subscript 30) storage for 30 days at different relative humidities. A = coarse sieve fraction, B = fine sieve fraction, C = spray-dried. Subscripts: a = stored in sealed bottles over P_2O_5; b–e were stored open at b = 23%, c = 52%, d = 75%, and e = 93% RH. XRPD was not performed on coarse or fine sieve fractions after storage at different relative humidities. No diffraction pattern is shown for spray-dried sucrose, C_{30e}, which dissolved in its own sorbed moisture at 93% RH.

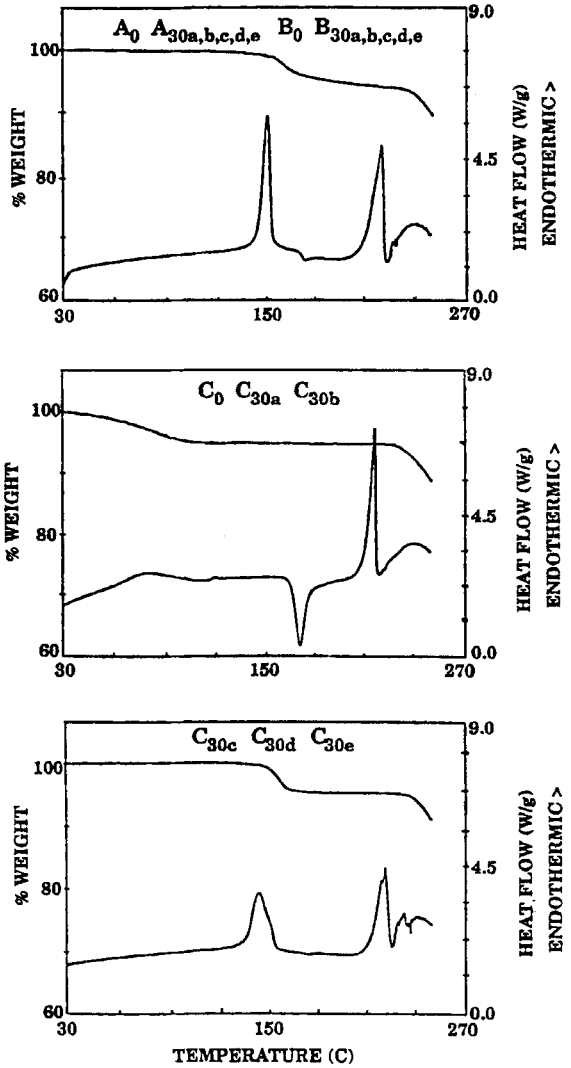

Figure 4. Typical TGA and DSC thermograms of lactose before (subscript 0) and after (subscript 30) storage for 30 days at different relative humidities. A = coarse sieve fraction, B = fine sieve fraction, C = spray-dried. Subscripts: a = stored in sealed bottles over P_2O_5; b–e were stored open at b = 23%, c = 52%, d = 75%, and e = 93% RH. Only TGA was performed on A and B before and after exposure at different RH.

days, dissolved in its own sorbed moisture, due to its known hygroscopicity and critical relative humidity of 84% (23,24). Lactose (monohydrate) and trehalose (dihydrate) showed water contents in agreement with theory (theoretical percent water in lactose and trehalose = 5.0% and 9.5%, respectively) and compendial specifications (25). Although lactose failed to sorb or lose water significantly under any of the storage conditions tested over a 30-day period, trehalose (especially fine fraction B) showed a tendency to sorb more water at increased relative humidities (Table 2). This tendency of trehalose appeared to involve surface moisture associated with an increased weight fraction of smaller crystals (fines) in the

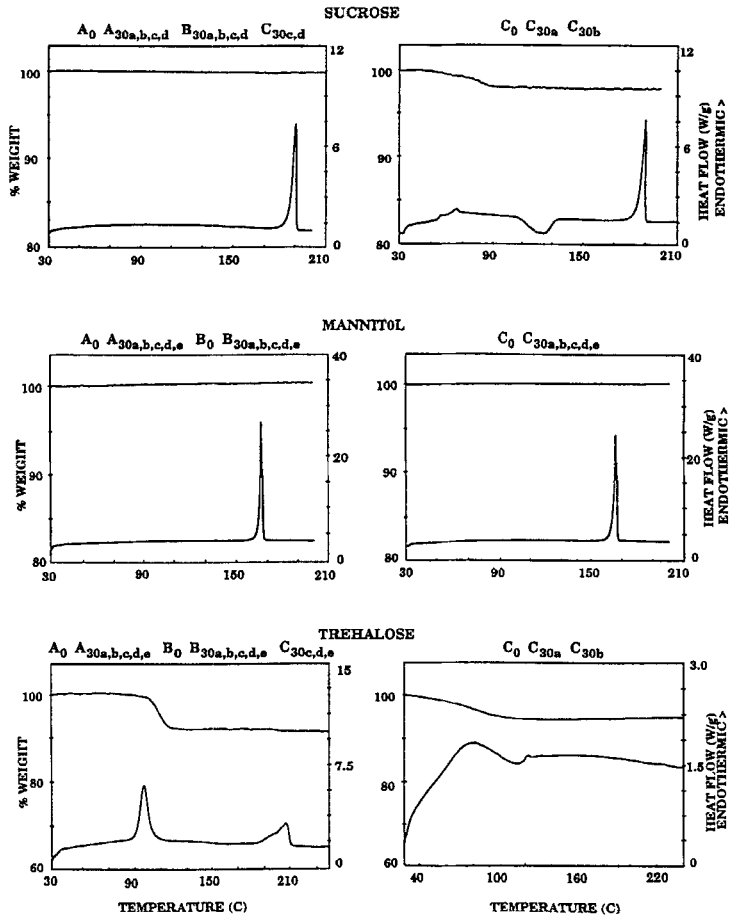

Figure 5. Typical TGA and DSC thermograms of trehalose, sucrose, and mannitol before (subscript 0) and after (subscript 30) storage for 30 days at different relative humidities. A = coarse sieve fraction, B = fine sieve fraction, C = spray dried. Subscripts: a = stored in sealed bottles over P_2O_5; b–e were stored open at b = 23%, c = 52%, d = 75%, and e = 93% RH. The second (melting) endotherm for trehalose, shown in the top left frame, was poorly reproducible for spray-dried material that had recrystallized on storage ($C_{30c,d,e}$). Sucrose A_{30e}, B_{30e}, and C_{30e} dissolved in sorbed moisture at 93% RH. Only TGA was performed on A and B before and after exposure at different RH.

trehalose as originally supplied [Fig. 6(e)] (26). Similarly, and because of their larger specific surface, both fine sieve fractions, B, of lactose and trehalose sorbed significantly more water than the respective coarse fractions, A, after storage at relative humidities ≥ 52% (26).

DSC results for the crystalline sieve fractions of all four sugars showed that there were no significant differences between sieve fractions A and B with respect to either the temperatures or the magnitudes of recorded thermal transitions. Lactose thermograms showed a dehydration endotherm (120–160°C), a small recrystallization exotherm at 172°C, and a melting endotherm (in agreement with literature reports [27,28]) between 190°C and 225°C. Trehalose thermograms showed dehydration and melting endotherms at 97°C and 207°C, respectively (Table 2 and Fig. 5). Our result for the melting temperature of trehalose was consistent with the value of 203°C reported by Slade and Levine (29), but not with the value of 96–97°C reported in the Merck Index (30). However, when trehalose was observed with a hot-stage microscope under cross polarized filters, some birefringence was lost at approximately 92°C, as confirmed recently by Taylor et al. (31). Sucrose thermograms (Fig. 5) showed only a melting endotherm between 175°C and 200°C, with no dehydration event or detectable weight loss at temperatures up to 160°C (Table 2; anhydrous sucrose melts at 188°C). Mannitol behaved similarly (Fig. 5), showing only a sharp melting endotherm between 150°C

Table 2

Moisture Contents of Sugars, Before and After Storage at 25°C and Different Relative Humidities, as Determined by Thermogravimetric Analysis

Sample	Temperature range used for weight loss	% Moisture Content[a]				
		Initial[b]	23% RH	52% RH	75% RH	93% RH
Lactose	31–200°C					
Coarse fraction, A		4.83 (0.14)	4.91 (0.05)	4.95 (0.05)	4.84 (0.03)	4.65 (0.03)
Fine fraction, B		5.34 (0.12)[c]	5.29 (0.19)	5.21 (0.12)	5.35 (0.05)	5.33 (0.02)
Spray-dried, C		2.58 (0.15)	5.08 (0.34)	4.29 (0.37)	4.94 (0.30)	5.02 (0.43)
Trehalose	31–180°C					
Coarse fraction, A		9.12 (0.16)	9.12 (0.14)	9.21 (0.07)	9.33 (0.09)	9.30 (0.04)
Fine fraction, B		8.87 (0.06)[c]	8.95 (0.04)	9.44 (0.19)	9.53 (0.05)	9.54 (0.16)
Spray-dried, C		4.09 (0.27)	5.62 (0.68)	9.50 (0.08)	9.42 (0.17)	9.46 (0.13)
Sucrose	31–160°C					
Coarse fraction, A		ND[d]	ND[d]	ND[d]	ND[d]	e
Fine fraction, B		ND[cd]	ND[d]	ND[d]	ND[d]	e
Spray-dried, C		2.36 (0.19)	4.57 (0.33)	1.82 (1.35)	ND[d]	e
Mannitol	31–200°C					
Coarse fraction, A		ND[d]	ND[d]	ND[d]	ND[d]	ND[d]
Fine fraction, B		ND[cd]	ND[d]	ND[d]	ND[d]	ND[d]
Spray-dried, C		ND[d]	ND[d]	ND[d]	ND[d]	ND[d]

[a] Values are mean ± (experimental range), n = 3; % moisture = (weight loss/initial weight) × 100.
[b] Stored in sealed bottles over P_2O_5 and tested on day 0 and day 30.
[c] Mean (sample SD, n = 5) moisture contents of fine fractions as determined by Karl Fischer analysis were 5.1(0.8), 8.4(0.1), 0.03(0.06), and 0.02 (0.06) for lactose, trehalose, sucrose, and mannitol, respectively.
[d] No detectable weight loss.
[e] Sucrose dissolved in sorbed moisture at this RH.

Table 3

Median Aerodynamic Diameters[a] for Spray-Dried Sugars Based on Volume Distributions, as Determined by Aerosizer® with Aerodisperser™

Sample	Median Aerodynamic Diameter (μm)	Geometric Standard Deviation
Lactose	5.56 (0.25)	1.64 (0.26)
Trehalose	5.23 (0.47)	1.64 (0.39)
Sucrose	12.53 (2.72)	2.18 (0.34)
Mannitol	4.79 (0.09)	1.46 (0.02)

[a] Values are averages (experimental range) of two determinations of the median aerodynamic diameter, where aerodynamic diameter = [equivalent spherical diameter × (density)$^{1/2}$]. Density was determined by helium pycnometry for spray-dried lactose, trehalose, sucrose, and mannitol as 1.54, 1.52, 1.53, and 1.43 g/cm^{-3}, respectively.

and 175°C, with no dehydration event or detectable weight loss at temperatures up to 200°C. DSC results for both these anhydrous sugars were consistent with literature reports (28,32). The microscopic size and appearance of the crystals of all sugars (Fig. 6) was unaffected by 30 days of storage at different humidities (with the exception of sucrose, which dissolved in sorbed moisture at 93% RH). Thus, all of these crystalline sugars appeared to be thermodynamically stable when stored for 30 days at 25°C at a range of relative humidities.

Spray-Dried Sugars

The spray-drying conditions for each sugar are listed in Table 2. Spray drying of particulates small enough for inhalation purposes required a high-collection-efficiency, small-droplet spray-dryer. Many commercial devices are inappropriate because their droplet dispersions are too large, thus demanding small solute concentrations (the alternative means of producing small particulates); this in turn reduces efficiency by requiring long spray-drying times. The Yamato ADL31 and the nominal spray-drying conditions for use with the sugars examined in the study were chosen on the basis of work reported previously (33). Nominal spray-drying conditions were held con-

Table 4

Summary of Differential Scanning Calorimetry Results for Lactose

	Glass Transition[b]		Dehydration[b]			Recrystallization[b]			Melting[b]		
Sample[a]	Onset (°C)	Midpoint (°C)	Onset (°C)	Peak (°C)	ΔH (J/g)	Onset (°C)	Peak (°C)	ΔH[c] (J/g)	Onset (°C)	Peak (°C)	ΔH (J/g)
A_0	ND[d]	ND[d]	144.8 (0.3)	149.5 (0.3)	125.1 (6.5)	ND[d]	ND[d]	ND[d]	211.8 (1.5)	219.2 (0.2)	135.0 (6.5)
B_0	ND[d]	ND[d]	143.6 (0.3)	148.9 (0.1)	133.2 (8.0)	ND[d]	ND[d]	ND[d]	207.7 (3.9)	217.1 (0.7)	127.4 (3.2)
C_0, C_{30a}	115.7 (1.3)	116.9 (0.9)	ND[d]	ND[d]	ND[d]	166.8 (0.5)	172.3 (1.0)	−111.4 (6.9)	211.5 (0.2)	215.8 (0.2)	132.8 (6.5)
C_{30b}	115.1 (0.9)	117.1 (0.1)	ND[d]	ND[d]	ND[d]	165.9 (0.1)	171.4 (0.0)	−106.4 (7.7)	211.4 (0.1)	215.5 (0.3)	127.4 (5.4)
C_{30c}	ND[d]	ND[d]	125.6 (2.4)	136.3 (2.3)	97.3 (6.3)	ND[d]	ND[d]	ND[d]	213.7 (0.7)	218.8 (0.8)	95.0 (12.7)
C_{30d}	ND[d]	ND[d]	134.5 (1.8)	142.0 (3.2)	125.2 (11.9)	ND[d]	ND[d]	ND[d]	214.0 (6.8)	219.2 (4.5)	99.2 (6.8)
C_{30e}	ND[d]	ND[d]	139.2 (1.1)	145.0 (2.1)	121.4 (18.4)	ND[d]	ND[d]	ND[d]	212.3 (1.7)	217.7 (0.9)	119.5 (6.7)

[a] A = coarse fraction, B = fine fraction, and C = spray-dried. Subscripts 0 and 30 refer to before and after 30 days of storage at 25°C, in sealed bottles over P_2O_5 (a), 23% RH (b), 52% RH (c), 75% RH (d), and 93% RH (e).

[b] Values are mean ± (experimental ranges), $n = 3$.

[c] Negative values are exothermic enthalpies.

[d] No detectable thermal event.

stant and close to optimal for maximum production efficiency of particles less than 5 μm (33). Reductions in the nominal inlet temperature of 150°C (to 125°C) were required in the case of sucrose and trehalose, which formed crusty masses at 150°C instead of the free-flowing material collected with the reduced inlet temperature. The aspirator setting was also reduced for trehalose, as described in Table 1. All spray-dried sugars were collected as dry, free-flowing powders, at rates ≥ 1 g/min, by cyclone separation. Table 3 shows the apparent particle-size distributions of each of the four products in terms of median aerodynamic diameters and geometric standard deviations. Results are expressed as aerodynamic diameters because of the common association of this term with the prediction of aerosol deposition in the lung (34). With the exception of sucrose, which formed cohesive agglomerates (apparent median aerodynamic diameter = 12.5 μm; agglomerates failed to deaggregate even under high-shear conditions [21]), product median aerodynamic diameters were approximately 5 μm. Theoretically, spheres with identical diameters should vary in terms of their aerodynamic diameter if their densities are different (aerodynamic diameter = equivalent spherical diameter × density$^{1/2}$). Table 3 shows that the median aerodynamic diameters of lactose, trehalose, and mannitol ranked in order of product density, as predicted.

Lactose, sucrose, and trehalose were all obtained in the amorphous form following spray-drying, as expected (16,35). None of these products showed birefringence under cross polarized filters, and all three displayed a typical amorphous "halo" after powder diffraction (Figs. 2 and 3). Conversely, spray-dried mannitol was birefringent under crossed polars and highly crystalline according to XRPD (Fig. 3). Although spray drying is often believed to offer insufficient opportunity for nucleation and crystal growth, because of short average droplet drying times (approximately 11 sec in the Yamato ADL31 under the operating conditions shown in Table 1), there was clearly sufficient time to enable crystallization of mannitol, an excipient that is known to crystallize readily in other circumstances (36).

Attempts were made to quantify the amorphous content of the spray-dried mannitol before and after storage at different R.H. Assuming that the original material purchased from Sigma was effectively 100% crystalline, the decreases seen in peak height following XRPD were probably due to crystallite-size effects (37), as shown for lactose in Fig. 2. There was no evidence, from the diffrac-

Table 5

Summary of Differential Scanning Calorimetry Results for Trehalose

	Glass Transition[b]		Dehydration[b]			Melting[b]		
Sample[a]	Onset (°C)	Midpoint (°C)	Onset (°C)	Peak (°C)	ΔH (J/g)	Onset (°C)	Peak (°C)	ΔH (J/g)
A_0	ND[d]	ND[d]	91.2 (0.3)	97.7 (0.1)	206.4 (12.0)	210.5 (4.4)	207.4 (1.4)	130.5 (5.0)
B_0	ND[d]	ND[d]	89.8 (0.1)	96.1 (0.4)	220.2 (3.9)	204.9 (0.4)	207.9 (0.3)	141.2 (4.6)
C_0, C_{30a}	119.2 (0.5)	120.3 (0.2)	ND[d]	ND[d]	ND[d]	ND[d]	ND[d]	ND[d]
C_{30b}	118.7 (0.8)	120.1 (0.4)	ND[d]	ND[d]	ND[d]	ND[d]	ND[d]	ND[d]
C_{30c}	ND[d]	ND[d]	97.7 (0.3)	101.4 (2.4)	165.6 (9.0)	208.8 (6.8)	212.7 (2.5)	52.2[e] (66.4)
C_{30d}	ND[d]	ND[d]	96.8 (0.4)	100.8 (0.7)	170.3 (27.9)	208.1 (4.0)	211.9 (0.7)	53.5[e] (73.1)
C_{30e}	ND[d]	ND[d]	97.2 (0.4)	100.6 (1.3)	195.4 (24.7)	ND[d]	ND[d]	ND[d]

[a] A = coarse fraction, B = fine fraction, and C = spray-dried. Subscripts 0 and 30 refer to before and after 30 days of storage at 25°C, in sealed bottles over P_2O_5 (a), 23% RH (b), 52% RH (c), 75% RH (d), and 93% RH (e).
[b] Values are mean ± (experimental ranges), $n = 3$.
[c] Negative values are exothermic enthalpies.
[d] No detectable thermal event.
[e] Melting behavior of recrystallized trehalose was not reproducible.

Table 6

Summary of Differential Scanning Calorimetry Results for Sucrose

	Glass Transition[b]		Dehydration[b]			Melting[b]		
Sample[a]	Onset (°C)	Midpoint (°C)	Onset (°C)	Peak (°C)	ΔH (J/g)	Onset (°C)	Peak (°C)	ΔH (J/g)
A_0	ND[d]	ND[d]	ND[d]	ND[d]	ND[d]	186.8 (0.2)	189.5 (0.2)	123.4 (2.6)
B_0	ND[d]	ND[d]	ND[d]	ND[d]	ND[d]	188.1 (0.0)	189.8 (0.2)	130.2 (1.9)
C_0, C_{30a}	60.2 (2.8)	62.7 (1.0)	111.7 (13.7)	126.1 (5.2)	−82.9 (11.3)	186.5 (1.6)	188.8 (0.2)	120.8 (7.1)
C_{30b}	ND[d]	ND[d]	116.4[e] (4.4)	123.3[e] (1.0)	−18.6[e] (27.8)	187.1 (1.5)	188.7 (1.0)	109.6 (26.9)
C_{30c}	ND[d]	ND[d]	ND[d]	ND[d]	ND[d]	183.1 (1.4)	185.8 (0.5)	107.2 (3.0)
C_{30d}	ND[d]	ND[d]	ND[d]	ND[d]	ND[d]	182.4 (0.7)	185.2 (1.2)	102.4 (6.8)

[a] A = coarse fraction, B = fine fraction, and C = spray-dried. Subscripts 0 and 30 refer to before and after 30 days of storage at 25°C, in sealed bottles over P_2O_5 (a), 23% RH (b), 52% RH (c), and 75% RH (d).
[b] Values are mean ± (experimental ranges), $n = 3$.
[c] Negative values are exothermic enthalpies.
[d] No detectable thermal event.
[e] Values are mean ± (experimental range), $n = 2$.

Table 7

Summary of Differential Scanning Calorimetry Results for Mannitol

	Melting[b]		
Sample[a]	Onset (°C)	Peak (°C)	ΔH (J/g)
A_0	164.8 (0.1)	166.9 (0.4)	268.5 (22.4)
B_0	165.1 (0.4)	167.4 (1.1)	282.6 (11.5)
C_0, C_{30a}	163.9 (0.5)	166.3 (0.4)	297.8 (53.1)
C_{30b}	164.1 (0.1)	166.3 (0.2)	276.5 (25.1)
C_{30c}	164.2 (0.5)	166.3 (0.2)	276.5 (25.1)
C_{30d}	164.0 (0.2)	166.6 (0.5)	283.0 (28.3)
C_{30e}	164.4 (0.2)	166.3 (0.3)	275.4 (43.9)

[a] A = coarse fraction, B = fine fraction, and C = spray-dried. Subscripts 0 and 30 refer to before and after 30 days of storage at 25°C, in sealed bottles over P_2O_5 (a), 23% RH (b), 52% RH (c), and 75% RH (d).

[b] Values are mean ± (experimental ranges), $n = 3$.

tograms of spray-dried mannitol, of an amorphous background (compare A and B to C for mannitol in Fig. 3). Moreover, the spray-dried material showed statistically identical, storage- and humidity-independent melting endotherms (Fig. 5, Table 7; no significant difference, $p >> 0.05$ by single-factor ANOVA, between melting endotherms for the crystalline starting materials and mannitol type C, stored under any study conditions). There were no shifts from baseline in the thermograms for type C other than the melting endotherm (Fig. 5), and TGA profiles were also unchanged by spray-drying or storage conditions (Table 2). Exposure of the spray-dried mannitol to high humidity in a microcalorimeter (VTI, Hialeah, FL) also failed to induce any observable recrystallization event. Thus, mannitol type C in these studies was, to all intents and purposes, 100% crystalline.

The amorphous form collected following spray-drying of lactose, trehalose, and sucrose was unstable in the solid state at 25°C, reverting to the crystalline form (forming sintered masses) in all cases at RH ≥ 52%. Type C materials, showing an amorphous halo after XRPD (Figs. 2 and 3), produced diffraction patterns, following recrystallization, that were indistinguishable from those of the original starting materials, indicating the return of an identical crystal structure. In the case of lactose, because α and β isomers are known to have different crystal structures and diffraction patterns (the β form has a major peak at $2\Theta = 10°$ [38]), there was no evidence of crystalline β-lactose in the XRPD data for the recrystallized, spray-dried product. We estimated, based upon known mutarotation kinetics for lactose (39), and a solution-to-

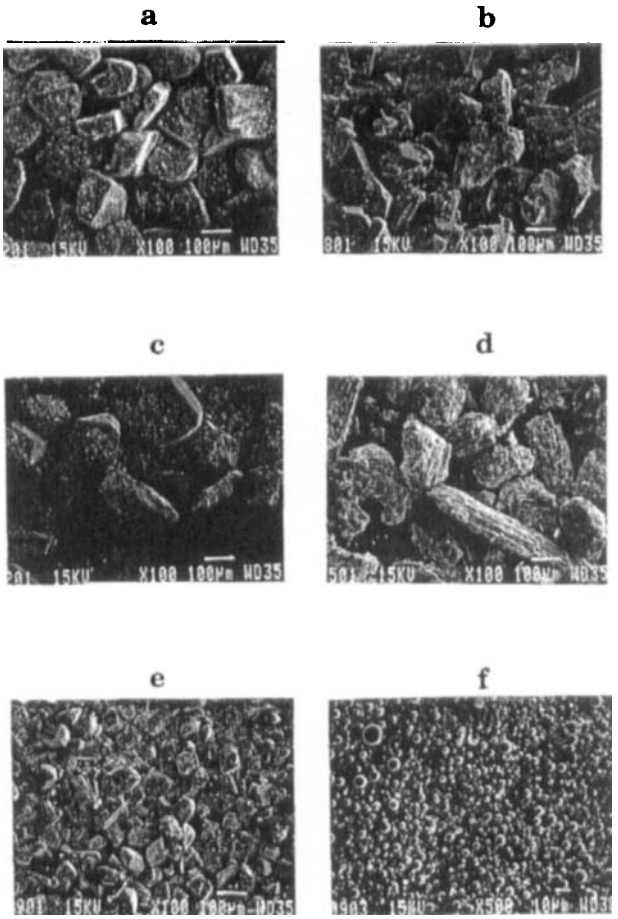

Figure 6. Scanning electron micrographs: (a–d) Coarse sieve fractions (type A) of lactose, trehalose, sucrose and mannitol, respectively (scale bar = 100 μm). (e) Fine sieve fraction (type B) of trehalose (scale bar = 100 μm). (f) Spray-dried mannitol (scale bar = 10 μm). All spray-dried sugars appeared similar to the mannitol shown in panel f immediately after collection.

dry-product time difference of approximately 45 min, that 5–7% of the β isomer was present in this spray-dried lactose. In samples stored at 52% and 75% RH, however, there was evidence of further α-to-β mutarotation in the solid state (Fig. 4 and below), even though XRPD data failed to show its existence. The XRPD similarity between lactose type A or B (α-form) and the recrystallized type C (Fig. 1) indicated either that there was not enough of the recrystallized β form for detection by XRPD, or that the β form had not recrystallized (in the presence of α-lactose), or both.

None of the sugars in this study, other than lactose, show mutarotation in solution, and thus, the spray-dried forms of trehalose, sucrose, and mannitol do not contain

mixtures of isomers. Consequently, interpretation of thermograms for these spray-dried sugars was simpler and is summarized in Tables 5, 6, and 7. The amorphous trehalose and sucrose (C_0,$C_{30a,b}$) showed weight loss beginning at approximately 31°C. Thus, moisture that was sorbed into the amorphous powders (Table 2) following spray-drying was loosely bound (26). By DSC, amorphous trehalose showed increasing heat uptake (climbing baseline), until the sorbed water was driven off, followed by a glass transition at about 120°C (Table 5), and showed no melting endotherm (Fig. 5; typical of an amorphous solid, behaving as a supercooled liquid). There was no recrystallization when DSC was performed in unsealed sample pans, in contrast to a recent report (40), of spray-dried trehalose having recrystallized at 134°C during DSC in hermetically sealed pans. Water, which is retained during heating in hermetically sealed pans, can enhance molecular mobility (26,29), and this may have enabled recrystallization under those circumstances. Amorphous sucrose (Table 6), on the other hand, showed a glass transition during the evolution of sorbed water (approximately 60°C), a recrystallization exotherm (confirmed by hot stage microscopy) at about 125°C, and a resultant melting endotherm at 180–190°C. Following moisture-induced recrystallization over a period of 30 days at RH $\geq$ 52%, the thermal behavior of sucrose and trehalose became typical of the original crystalline materials.

The interpretation of spray-dried lactose thermograms (before and after solid-state recrystallization during storage at 25°C) is complicated by the presence of a mixture of α and β isomers in the product; the ratio of the two is frequently variable in spray-dried lactose (41,42). Thus, the results shown in Fig. 4 and Table 4 for lactose type C may well be different if the ratio of the isomers in the sample is different. Although the onset of weight loss for amorphous, spray-dried lactose (Fig. 4, C_0,$C_{30a,b}$) was 30°C, the DSC results showed an initial broad endotherm corresponding to loss of sorbed moisture, a glass transition (T_g) at 117°C, an exothermic recrystallization event at 172°C, and a melting endotherm at 216°C. The value for T_g was as expected for this dry sugar after the loss of sorbed water (43–45). Following moisture-induced recrystallization, enthalpies of dehydration and melting (Table 4) were lower than the values expected for crystalline α-lactose monohydrate. This was indicative of variations in the content of the α form; the progressive decrease in the enthalpy of melting (Table 4, type C, with increasing RH to 75%) showed continuing mutarotation of lactose in the solid state at moderately high relative humidities (38). The sample stored at 93% RH, however, showed the increased stability of the α-lactose monohydrate once it achieved this crystalline form (melting enthalpy was closer to the original value for spray-dried lactose). At this higher humidity, recrystallization occurred rapidly, before any further mutarotation could occur in the amorphous form. Thus, in this sample stored at 93% RH, the content of α-lactose was similar to that of the original spray-dried product. Also, in the samples stored at 52% RH and 75% RH (Fig. 4), there was a small endotherm at around 230°C, the melting point of anhydrous β-lactose (27).

Spray-dried lactose, trehalose, and sucrose showed moisture uptake at all relative humidities, with or without changes in their physical form. At 23% RH, all three sugars showed increased moisture contents relative to the initial values (Table 2). However, no crystallization was detected in these samples by DSC (Figs. 4 and 5) or XRPD (Figs. 2 and 3) in the time frame of exposure (i.e., after 30 days).

Sugars as Excipients in Powder Inhalers

The work described here was initiated to dovetail with our investigations of crystalline drug carriers (with different interparticulate bonding strengths and/or electrostatic behavior [43]) and aerosolizable spray-dried excipients with the capacity to protect some proteins against denaturation during spray-drying (8). Differences in dissolution rates between amorphous and crystalline powders for inhalation are unlikely to produce different in vivo effects or absorption-vs.-time profiles because particles reaching the lung have such high surface-to-volume ratios that they dissolve rapidly, under sink conditions (34). Thus, at the outset, it seems possible to use either type of material. At present, lactose is the only acceptable carrier for dry-powder inhalation systems in the United States. However, all of the sugars obtained as crystalline sieve fractions in this investigation appeared to be suitable for admixture with micronized drugs for presentation in powder inhalers. Only crystalline sucrose, which dissolved in its own sorbed moisture at 93% RH, appeared to require extraordinary packaging precautions, in order to protect against moisture-induced formulation changes at high humidities. There was no evidence of mutarotation of α-lactose monohydrate at any storage humidity or duration tested (water contents and DSC results were unchanged), indicating the stability of its crystal structure at 25°C (mutarotation in the solid state requires molecular mobility). It remains to be seen whether all these sug-

ars will also offer the short-term moisture protection seen previously with aerosolization of albuterol sulfate in the presence of lactose at high humidities (46). In this case the drug formed cohesive agglomerates over a < 3-min time frame when aerosolized at high humidities in the absence of a lactose diluent (19). It is tempting to speculate that the capacity of different sugar carriers to sorb surface moisture at high humidities (Table 2) may be related to an ability to prevent or retard moisture-induced changes in drug detachment from the carriers during aerosolization.

The selection of alternative crystalline drug carriers requires consideration of several factors that are likely to affect powder-flow and drug-dispersion characteristics. Of these factors, particle-size distribution should be controlled no matter which material is chosen (21). Although crystal shape and surface morphology of carrier particles can be modified by careful choice of crystallization conditions (4), the formulator cannot control the underlying crystal structure. There were obvious differences in crystal habit and surface morphology between the sugars in this study (Fig. 6). Mannitol's flaky elongated crystals flowed more poorly than the smoother, more regularly shaped crystals of lactose, trehalose, or sucrose. This may cause powder-emptying problems when mannitol is used in some powder inhalers (47). Predicting which of these sugars is an "optimal" drug carrier with respect to blend stability and ease of drug dispersion during aerosolization requires further work beyond inspection of the SEMs shown in Fig. 6. Optimal combinations are likely to be drug dependent and drug:carrier-ratio dependent, and an experimental review of inhaler performance using a larger number of well-characterized excipients as carriers is therefore advisable during formulation development. It is likely that alternative sugars can be used to advantage in powder inhalers when a given drug blend with lactose has been shown to perform poorly (48).

Of the four spray-dried products tested in this study, only sucrose was intensely hygroscopic and difficult to deaggregate even under dry conditions. Both problems may prevent powder-inhaler development with this sugar unless a specific sucrose–drug molecular mixture is known to behave differently. The large median aerodynamic diameter shown in Table 3 for spray-dried sucrose was a direct result of inadequate de-agglommeration during the powder aerosolization process necessary for particle sizing with the Aerosizer/Aerodisperser (scanning electron micrographs [SEMs] of all spray-dried products were similar to that shown in Fig. 6(f)]. The Aerosizer/Aerodisperser, under fairly low shear conditions (Table 3), easily redispersed spray-dried lactose, trehalose, and mannitol. Thus, within the constraints described below, each of these three sugars could feasibly be used as a spray-drying excipient in powder inhalers.

Spray-dried sugars stored at high humidities showed predictable recrystallization changes over time, with the exception of mannitol. Mannitol appeared to crystallize completely during the brief (11 sec) spray-drying period prior to collection and storage. Although its behavior with co-spray-dried solutes was not studied, its capacity to form stable, humidity-independent crystallites during spray-drying was unique among the sugars studied. Mannitol is a non-reducing sugar and, as we have discussed above, our efforts to show the presence of even small amounts of amorphous content in the spray-dried bulk were unsuccessful. Mannitol should certainly be studied further as a diluent for robust proteins and peptides during drying and storage (development of crystalline formulations for powder inhalers may be easier than that of their amorphous counterparts, both from a packaging and aerosolization point of view). However, it is unlikely that an excipient that crystallizes during drying can act as a protectant for labile proteins (14) and drug-containing, spray-dried respirable formulations (Table 3). In addition, these formulations are likely to include at least some amorphous content.

Amorphous products such as spray-dried trehalose and lactose formed sintered masses over 30 days at humidities > 23% and, although the kinetics of recrystallization were not investigated in the present study, such changes can be rapid and must not be allowed to occur in powder-inhaler formulations. Thus, both the powder packaging and water content of inlet air to the inhaler should be controlled for such products. Provided this can be achieved, and thus powder deaggregation made to occur reproducibly, the choice of a particular sugar as an excipient during spray-drying may well depend upon its stabilizing capacity (against protein denaturation and/or chemical degradation). In this context, lactose may be unsuitable because it may react with some proteins while in solution prior to and during drying (49). Of the sugars we studied lactose was the only reducing sugar (50). Lactose has the further disadvantage in that its spray-dried products are likely to contain mixtures of both α and β isomers. This is irrespective of the α/β ratio of the starting material (closure of the ring-opened aldehyde form occurs after free rotation of the 1′–2′ bond (Fig. 1) in the solution to be dried (41,50). The ratio of α to β lactose in the product can only be considered constant if the solution that was spray-dried has either stood for 6–7 mutaro-

tation half-lives, which is about 17 hr at 20°C (50). Alternatively, if the spray-drying conditions are carefully controlled and the product is kept dry, further mutarotation in the solid state can be prevented. Spray-dried sucrose (difficult to aerosolize) and lactose (reactivity during drying) may not be good excipient candidates during powder-inhalation-drug development. Mannitol may also be unsuitable because a sugar's protein-stabilizing capacity is believed to require the presence of its amorphous form, with its glass-transition temperature in the presence of any sorbed moisture having to exceed the storage temperature (14). Trehalose, with a high glass-transition temperature in its dry state, and a tendency to retain relatively large amounts of moisture without recrystallizing, may have excellent stabilizing properties in this regard (51).

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