Thermochimica Acta
Elsevier Publishing Company, Amsterdam
Printed in Belgium

CALORIMETRIC MEASUREMENT OF THE HEAT OF DESORPTION OF WATER VAPOR FROM AMORPHOUS AND CRYSTALLINE LACTOSE

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(Received June 25th, 1970)

ABSRACT

The heat required to release and vaporize bound H_2O from crystalline α-lactose monohydrate and from lactose glass, as determined by differential scanning calorimetry is 12.3 ± 0.7 and 10.8 ± 0.5 kcal·mole^{-1} of H_2O, respectively. Water vapor sorption by anhydrous α-lactose leads to the formation of the α-monohydrate. The isotherm, obtained gravimetrically for this process is of Langmuir type. β-Lactose is completely non-hygroscopic below 97% relative humidity. Thereafter, it sorbs H_2O rapidly to form a concentrated solution wherein the lactose is capable of mutarotation. Densities of lactose forms determined pycnometrically by helium displacement are: 1.535 g/cm^3 for α-lactose·H_2O; 1.547 g/cm^3 for anhydrous α-lactose; and 1.576 g/cm^3 for β-lactose.

INTRODUCTION

During research on the physicochemical aspects of water removal from milk, we were led into a study of the relative energy requirements for dehydration of the individual chemical constituents of milk. The first component studied was lactose in its various solid state forms.

As a solid, lactose exists either in one of two crystalline forms or in a glassy amorphous state. These three forms differ widely in their water binding capacities. The two crystalline forms exhibit very limited hydration with α-lactose binding one water molecule per lactose molecule to form the commonly encountered α-lactose monohydrate, while β-lactose exists as a stable anhydride[1].

Water vapor sorption isotherms for lactose glass at several temperatures have been published[2,3]. At low relative pressure, $P/P_0 < 0.15$, little water is sorbed by the glass, but as the relative pressure is increased the lactose becomes extremely hygroscopic reaching a maximum weight at P/P_0 values between 0.4 and 0.6, depending upon the temperature at which the sorption isotherm is measured. Following this maximum weight a discontinuity is observed in the sorption curve, and water is desorbed from the lactose while a constant water vapor pressure is maintained in the system.

This paper presents thermal data related to the sorption of water by the various forms of lactose. In addition, complete isotherms describing the hydration processes

are given along with the measured densities of the various crystalline forms of lactose under investigation. From a consideration of these data, factors responsible for the markedly different water sorption properties of the various solid forms of lactose are inferred in terms of current theories of the lattice energy of crystalline hydrates[4,5].

EXPERIMENTAL

*Materials**

The crystalline α- and β-lactose examined in this study were reagent grade materials used as obtained from chemical supply houses (α-lactose, Baker Analyzed Reagent and Fisher Certified Reagent, β-lactose, Eastman Kodak white label). Amorphous lactose was prepared by lyophilizing an aqueous lactose solution, the accepted method for preparing lactose in this form[6].

Calorimetric standards used were: indium, lead, and tin (Perkin–Elmer), benzoic acid (James Hinton, 99.99% zone refined), naphthalene (James Hinton, 99.999% zone refined), and anthracene (James Hinton, 99.999% zone refined).

Calorimetry

The Perkin–Elmer Model DSC-1B differential scanning calorimeter was employed to observe the thermal properties of the various lactose modifications in the temperature interval between 0 and 260 °C. Calorimetric analyses were performed to determine the heats of dehydration of α- and amorphous lactose and the heats of fusion of α- and β-lactose. Experimental conditions such as sample size and container as well as the temperature interval scanned were varied in accord with the aims of the particular experiment.

In determining the heat of desorption of the bound water in α-lactose·H_2O, 5–10 mg samples, weighed with the Cahn Model M-10 electrobalance, were packed in the standard crimped aluminum sample pans supplied by the Perkin–Elmer Corporation and scanned at a programmed rate of 10°/min from 35 to 170 °C. These crimped sample containers are not hermetically sealed and allow for rapid removal of H_2O vapor from the calorimeter cell in a stream of flowing nitrogen. This mode of operation was satisfactory with the crystalline α-lactose·H_2O as there was no removal of bound water at temperatures lower than 90–95 °C nor is there any measurable adsorption of water vapor by α-lactose·H_2O at ambient temperature even at higher relative humidities.

In studying the dehydration of the lactose glass, precautions were necessary to avoid water vapor transfer between the sample and the atmosphere prior to scanning in the calorimeter. This was accomplished by rapidly cooling the sample, in a crimped container, to 0 °C in the calorimeter cell prior to beginning the scan. Loss of moisture from the sample was negligible at this temperature and additional H_2O uptake would not occur in the flowing N_2 atmosphere.

*Mention of brand or firm names does not constitute an endorsement by the Department of Agriculture over others of a similar nature not mentioned.

The influence of atmosphere control on the dehydration of α- and amorphous lactose was studied by scanning 3–4 mg samples in hermetically sealed capsules (Perkin–Elmer volatile sample sealer accessory).

The effect of moisture sorption on the water binding properties and the crystallinity of the lactose glass was studied. Samples of the glass were equilibrated with H_2O vapor in a humidistat at 70% relative humidity (r.h.), at ambient temperature (20–23 °C). At different time intervals samples were removed and subjected to differential scanning calorimetry and thermogravimetric analysis.

Heats of fusion of α- and β-lactose were determined using 2–2.5 mg samples heated at 10°/min in crimped containers.

In all scans with the calorimeter no diluents were used and empty containers and covers identical to those containing the samples were used for reference purposes.

The temperature scale of the DSC-1B was calibrated as directed in the manufacturer's instruction manual using the melting points of high purity samples of indium, lead, and tin. Calibration of the electrical output signal from the calorimeter for quantitative evaluation of the recorder tracings was performed by correlating the area under the endothermic peak for the melting of indium metal with its known heat of fusion ($\Delta H_f = 6.79$ cal/g). Peak areas were measured with a polar planimeter (Keuffel and Esser Company, K and E 4236). Measurements of ΔH_f for benzoic acid, naphthalene, and anthracene were made to check the DSC-1B and its calibration. These materials were scanned in sealed capsules to avoid errors from sublimation. The ΔH_f values were within experimental error of those found by Hampson and Rothbart[7] and other values from the literature as tabulated by them.

Thermogravimetric analysis

Thermogravimetric analyses (TGA) were performed on samples of α-lactose and hydrated lactose glass containing 3–10% H_2O, using the Cahn RG recording electrobalance with the "Little Gem" TGA Accessory of the Cahn Instrument Company. Data were plotted as mass against temperature with a Varian Model 100 X–Y recorder.

Samples were placed directly in the platinum bucket, supplied by the Cahn Instrument Co., and heated from ambient temperature (23–27 °C) up to 200 °C. No provisions are available for atmosphere control or desorbed vapor removal with the "Little Gem" accessory.

The heating rate was controlled manually with a laboratory variable transformer as suggested in the Cahn instruction manual. The variac was initially set at 40 V and turned up in 5 V increments every five minutes. A fairly uniform heating rate of 5°/min was routinely obtained by this method.

Sorption measurements

Water vapor sorption data for α- and β-lactose were determined gravimetrically using the Cahn RG recording electrobalance incorporated into a glass adsorption system, using methods described elsewhere[8]. Measurements were made in increments

of 0.05 P_0 over the entire adsorption isotherm at 24 °C. Prior to measuring water vapor sorption, the α-lactose was degassed at 110 °C to remove the water of hydration.

Density measurements

Lactose densities were determined by helium displacement using a volumetric gas-adsorption system as previously described[9]. Measurements were made on crystalline α- and β-lactose and anhydrous α-lactose prepared by outgassing the hydrate under high vacuum at 135 °C. The anhydrous α-lactose was not allowed to come into contact with the air so as to prevent conversion back to the monohydrate.

RESULTS

Calorimetry

Representative calorimetric scans for α-lactose monohydrate are shown in Figs. 1 and 2, corresponding to data obtained with crimped and sealed samples, respectively.

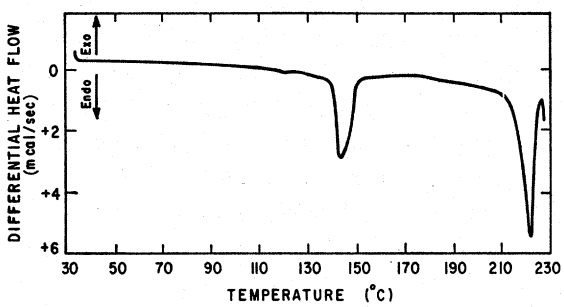

Fig. 1. DSC scan for α-lactose·H_2O heated in a crimped pan at 10 °/min, sample mass: 8.500 mg.

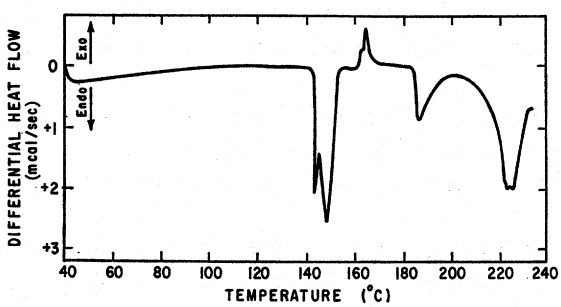

Fig. 2. DSC scan for 4.425 mg α-lactose·H_2O heated at 10°/min in a sealed container.

When heated in a crimped sample container (Fig. 1), α-lactose·H_2O exhibits an endothermic dehydration peak beginning at 97 °C, reaching a maximum at 144 °C and returning to the baseline at 167 °C. A second endothermic peak corresponding to the melting of α-lactose begins at 202 °C, reaches a maximum at 223 °C and returns to the baseline at 227 °C. At higher temperatures the lactose rapidly charred and de-

composed. Calculation of the heat of desorption of bound water, ΔH, from the area under the first endotherm in this and similar scans yields a value of $\Delta H_{des} = 12.3$ kcal/mole, with an average deviation of ± 0.7 kcal/mole, in twenty-seven separate determinations. The heat of fusion, ΔH_f^{α}, calculated from the second endothermic peak is 35.9 cal/g with an average deviation of ± 1.7 cal/g in six determinations.

Heating the α-lactose$\cdot H_2O$ in a sealed capsule (Fig. 2) does not yield a single endothermic dehydration peak. The observed peak is split and occurs simultaneously with an exothermic heat transfer. Two additional endothermic peaks are observed at higher temperatures. In parallel experiments, when samples were heated through appropriate shorter temperature ranges and the capsules punctured at suitable intervals to allow vapor escape, it was determined that no mass loss is involved in either of these latter endotherms.

Results obtained with β-lactose (Fig. 3) indicated no thermal transitions occurring below 220 °C. The single endothermic peak beginning at 220 °C, reaching

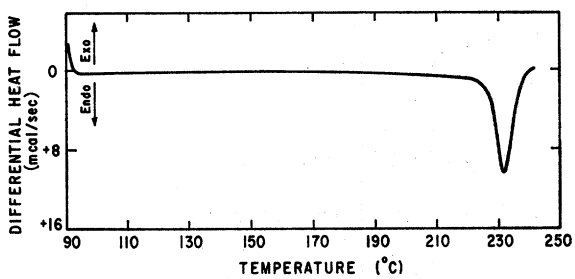

Fig. 3. DSC scan for 4.115 mg β-lactose heated at 10°/min in a sealed container.

its maximum height at 232 °C, and returning to the baseline at 241 °C, corresponds to the melting of β-lactose. The heat of fusion ΔH_f^{β}, calculated from this and similar scans is 48.7 cal/g with an average deviation of ± 1.3 cal/g in three determinations.

Lactose glass, when heated in a crimped sample container (Fig. 4) exhibits a broad dehydration endotherm over the entire temperature interval between 10 and

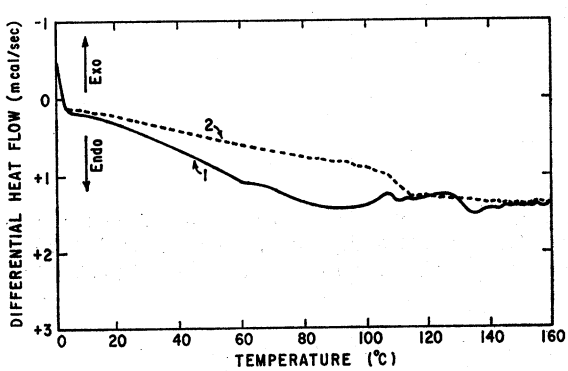

Fig. 4. DSC scan for lyophilized lactose heated at 10°/min in a crimped container. Curve 2 obtained on rescanning after dehydration.

120 °C. The second curve (Fig. 4) shown, was obtained on rescanning the same sample after H_2O desorption, and ΔH_{des} was determined from these curves together with a baseline curve previously established by scanning the empty pan and cover before filling with lactose. The computations were performed according to the procedure of Brennan *et al.*[10] which accounts for changes in specific heat as a result of the reaction. The computed heat of desorption is 10.8 ± 0.5 kcal/mole H_2O desorbed. On heating in a sealed container lactose glass exhibits only a single exothermic peak beginning at 97 °C with the peak maximum at 110 °C and end at 117 °C (Fig. 5). The area under this peak corresponds to an energy release of -4.7 kcal/mole of anhydrous lactose.

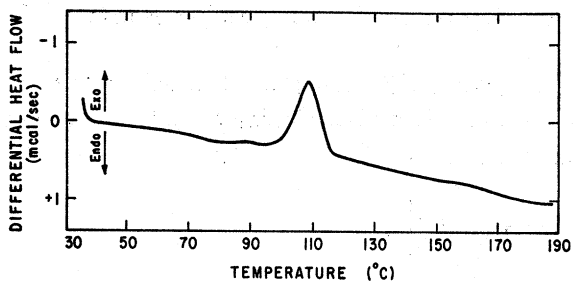

Fig. 5. DSC scan for 3.500 mg lyophilized lactose heated at 10°/min in a sealed capsule.

Calorimetric data showing the transformation of lactose glass into the crystalline α-monohydrate during the H_2O sorption process are given in Fig. 6. The DSC scans A through F correspond to samples of lactose held for increasing time intervals at

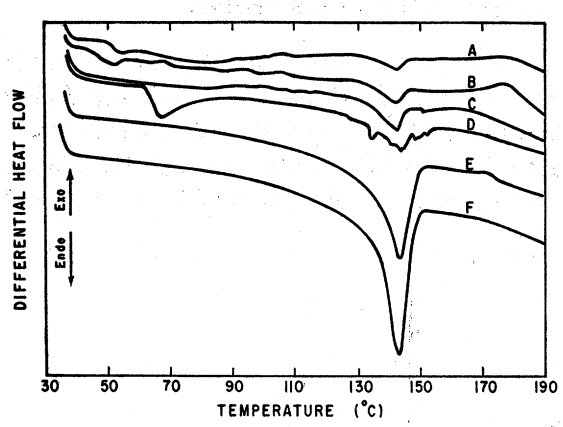

Fig. 6. Effect of H_2O sorption on amorphous lactose. Samples heated in crimped containers at 10°/min. A, 9.125 mg of original lyophilized lactose; B, 7.660 mg lactose, 7.31% H_2O; C, 7.460 mg lactose, 8.31% H_2O; D, 8.035 mg lactose, 9.26% H_2O; E, 6.775 mg lactose, 9.67% H_2O; F, 8.825 mg lactose, 9.04% H_2O.

70% r.h. at 23 °C. As the lactose is held longer in this humid environment, H_2O vapor sorption and desorption take place and the resultant scans become more like that of pure α-lactose·H_2O (compare curve F, Fig. 6, with Fig. 1).

Thermogravimetric analysis

TGA curves for α-lactose monohydrate and the glass are given in Fig. 7. The α-lactose·H_2O lost weight in a discrete temperature interval between 97 and 165 °C which corresponds closely to that observed on dehydration in the DSC-1B. The mass

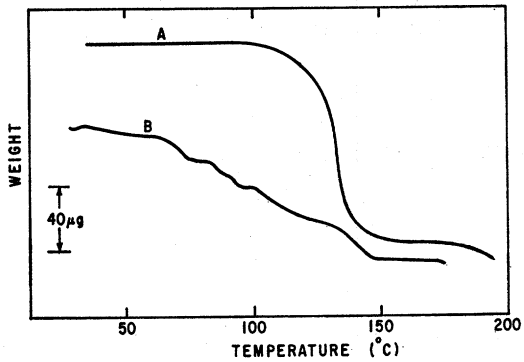

Fig. 7. TGA curves for (curve A) 2.458 mg α-lactose monohydrate and (curve B) 1.955 mg lactose glass.

loss in this interval, with five different samples, was very close to the theoretical value of 5% H_2O in lactose monohydrate. The TGA curve for the glass form however, shows a mass loss commencing at a lower temperature and extending over a wider range, indicating that the H_2O desorbed from the glass is not bound in the same manner as the water of hydration in the crystal. Analysis of amorphous lactose in various stages of hydration yielded a series of TGA curves describing the transition from amorphous to α-lactose, analogous to the sequence obtained with the DSC-1B (see Fig. 6).

Sorption data

The H_2O vapor sorption isotherm for anhydrous α-lactose is given in Fig. 8. This isotherm is of the Langmuir type representing limited adsorption and may be associated with the formation of the monohydrate, with no further H_2O sorption by the nonhygroscopic α-crystal. At very high relative pressure, $P/P_0 = 0.98$–1.00, water

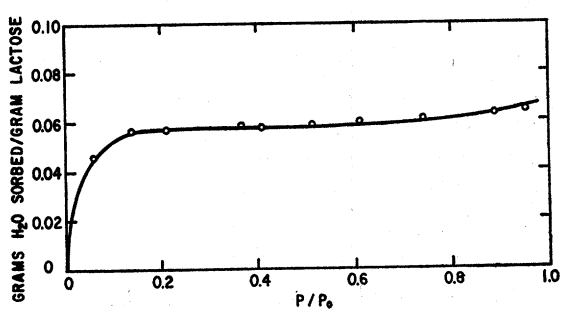

Fig. 8. H_2O sorption isotherm for anhydrous α-lactose at 24.2 °C.

is rapidly sorbed corresponding to a condensation and solution process. On redrying the sample, after being held at $P/P_0 = 1.0$, lactose glass was formed from the solution and this glass exhibited sorption properties typical of lactose glass[2,3].

No measurable H_2O sorption by β-lactose was observed below $P/P_0 = 0.97$. At higher humidities, β-lactose sorbs sufficient moisture to form a solution, undergo mutarotation, and subsequently desorb excess H_2O to form the crystalline α-lactose monohydrate.

Density data

The observed density values with the different forms of lactose were: α-lactose monohydrate, 1.535 g/cm^3; anhydrous α-lactose, 1.547 g/cm^3; and β-lactose, 1.576 g/cm^3.

DISCUSSION

The enthalpy measured in these experiments is the heat of desorption, ΔH_{des}, of H_2O from lactose which includes the enthalpy of dehydration or release of H_2O from the crystal lattice, and the enthalpy of vaporization of this liberated H_2O. These ΔH_{des} data may then be correlated with the sorption process by assuming that the enthalpy of sorption is equal in magnitude but of opposite sign to ΔH_{des}.

The calorimetrically determined ΔH_{des} of 12.3 kcal/mole of bound water in α-lactose$\cdot H_2O$ compares favorably with the heat of sorption of -11.5 kcal/mole of H_2O determined from multi-temperature sorption data[3]. These values are reasonable for a process involving hydrogen bond formation between water and a polar substrate, as Zettlemoyer and Narayan[11] have pointed out that -12.0 kcal/mole is an appropriate value for the heat of physisorption of H_2O on a polar surface through hydrogen bonding. The enthalpy value observed with the glass, $\Delta H_{des} = 10.8$ kcal/mole is somewhat lower and closer to the heat of vaporization of liquid H_2O, 10.5 kcal/mole at ambient temperature.

The absence of any great differences in ΔH_{des} for the lactose forms is indicative of the basic similarity in the way in which H_2O is bound in both instances, *i.e.*, through H-bonds. The results, however, do indicate that the H_2O molecules are probably more localized when bound to the α-hydrate than to the glass, which is to be expected as in the case of the hydrate, the H_2O becomes an integral part of the crystal lattice.

The exothermic peak observed on heating lactose in a sealed capsule (Figs. 2 and 5) may be associated with the solution of lactose in the released H_2O and the mutarotation of α-lactose to the β-form. Since removal of the H_2O vapor released from the hydrate is prevented in a sealed capsule, a concentrated lactose solution is formed similar to the glass, resulting in mutarotation[12]. The endothermic peaks observed at higher temperatures then correspond to the melting of α- and β-lactose, respectively. This interpretation is in accord with Sharp and Hand's[13] method for preparing β-lactose by heating α-hydrate in a closed container at 120–130 °C.

From the density data reported in this paper it is apparent that dehydration has

little effect of any significance on the density of α-lactose while the anhydrous β-lactose has a somewhat higher density value. These results are in general agreement with other data in the literature. Buma[14] determined the density of various milk components by centrifugation in CCl_4–liquid paraffin solutions and by air pycnometry and reported the following values for lactose forms: α-hydrate, 1.545 g/cm^3; α-anhydride (containing 0.17% H_2O), 1.545 g/cm^3; and β-anhydride, 1.59 g/cm^3. Buma concluded that the identity of the density values for α-lactose despite a 5% loss in mass due to water removal indicates substantial changes in the crystal lattice on desiccation. Herrington[15] studied the dehydration of lactose and reported on two variations of α-anhydride. Drying the α-hydrate under vacuum leads to an unstable, hygroscopic anhydride, termed α-desiccated, α_D, by Herrington. Crystallization of α-lactose from organic solvents yields a more stable anhydride, termed α-stable, α_s, by Herrington. Density values reported for these lactose forms are: α_D, 1.544 g/cm^3; α_s, 1.575 g/cm^3; and β-anhydride, 1.588 g/cm^3. From his density results and other data Herrington concluded that the product obtained on desiccation of α-lactose·H_2O *in vacuo* is not the stable crystalline anhydride, but it is a porous form obtained by loss of water from the crystal lattice without a complete rearrangement to form a stable crystal as with α_s. We are of the opinion that the similarity in values for the density of hydrated and anhydrous α-lactose, when considered in conjunction with the thermal data reported here, may aid in understanding why α-lactose exists as a hydrate while β-lactose does not.

In a series of papers[4,5] on crystalline hydrates, Ladd and Lee have formulated a mechanism for hydrate formation based on a modified Born–Haber cycle. Hydrate formation is considered in two steps. The anhydrous crystal structure is first expanded and gaseous water molecules accomodated so that the resulting structure is equivalent to that of the hydrate. The second stage is the expanded lattice–H_2O interaction which may be stabilized through covalent bonding ($CuSO_4 \cdot 5H_2O$), hydrogen bonding ($KF \cdot 2H_2O$), or ion–dipole interaction energy ($BaCl_2 \cdot 2H_2O$). The first stage is endothermic, ΔH_ζ, and the second is exothermic, ΔH_ϕ. The enthalpy of hydrate formation is thus: $\Delta H_w = \Delta H_\zeta + \Delta H_\phi$, and a hydrate will form if ΔH_w is negative, *i.e.*, the enthalpy of expansion is more than compensated by the water interaction enthalpy. This amounts to stating in effect that the reaction to form the hydrate is enthalpy controlled. Ladd and Lee[4] have computed values for ΔG_w and ΔS_w for a variety of crystal hydrates and concluded that in all cases considered, both the free energy and entropy changes are always negative. Reaction of the anhydrous crystal with water would be expected to lead to a decrease in entropy because H_2O molecules are losing translational and rotational degrees of freedom, or at least rotation is becoming hindered. The entropy change is always negative despite the fact that there is a positive entropy contribution due to expansion of the crystal with the lattice entropy of the hydrate being greater than the lattice entropy of the anhydride. It appears that the increase in lattice entropy can in no case compensate for the loss of entropy of water, hence for hydrate formation to occur, and ΔG_w to have a negative value, ΔH_w must be negative.

The mode of interaction between lactose and water is most likely through hydrogen bond formation which would occur with either α- or β-lactose. Actually Kabayama and Patterson[16] have pointed out that due to steric considerations, β-lactose is more hydrated through H-bond formation in solution than α-lactose, hence, equilibrium in solution favors the β-forms and ΔG of mutarotation to β is negative[17]. The water interaction enthalpies, ΔH_ϕ, for α- and β-lactose should therefore be the same or possibly ΔH_ϕ^β may even be more negative than ΔH_ϕ^α, hence the different hydration of α- and β-lactose crystals must be due to the ΔH_ζ term. The density values for anhydrous and hydrated α-lactose indicate little difference in molar volume, hence the anhydrous lattice can readily accomodate the gaseous H_2O molecules and ΔH_ξ^α is therefore smaller. β-Lactose with a higher melting point, and a greater density value is obviously more compact, and requires a greater expenditure of enthalpy, ΔH_ζ for expansion of the anhydrous lattice, thus preventing the formation of a hydrated crystalline β-lactose.

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