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Evaluation of heat-conduction microcalorimetry in pharmaceutical stability studies. III. Crystallographic changes due to water vapour uptake in anhydrous lactose powder

M. Angberg [1], C. Nyström [1] and S. Castensson [2]

[1] Department of Pharmaceutics, Uppsala University, Uppsala (Sweden) and [2] Kabi Pharmacia AB, Uppsala (Sweden)

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Summary

Microcalorimetry at 25.0 °C was used to investigate the incorporation of hydrate water in roller-dried anhydrous lactose that consisted of 31% α- and 69% β-lactose. Differential scanning calorimetry and water vapour uptake measurements were also performed. The samples were stored for various lengths of time in the range 33–94% relative humidity (RH) at ambient temperatures, before measurement. The anhydrous α-lactose can accommodate a water molecule to become α-lactose monohydrate. β-Lactose can only exist as the anhydrous form, but it can mutarotate to α-lactose and subsequently incorporate water, as shown by others. It was only at the highest humidity investigated (94% RH) that the mutarotation became an extensive process, although it was shown that the mutarotation did proceed at humidities lower than 94% RH and that it started before all the original anhydrous α-lactose had been converted to the monohydrate form. With the microcalorimetric technique, it was possible to monitor the incorporation of water after 1 day of storage at 58% RH. Furthermore, after 112 days of storage at 94% RH, it was still possible to detect a process, although the standard DSC instrument could not discriminate a change in the heat of dehydration level. It was concluded that, using microcalorimetry, a sensitive characterization of the incorporation of water in the lactose structure was possible and that microcalorimetry can be useful in solid state stability work.

Introduction

One technique extensively used in pharmaceutical preformulation and formulation work regarding solid material is differential scanning calorimetry (DSC). The measurements are usually performed rapidly, but the technique has its limitations. The instruments often have a low sensitivity and, in many stability studies, the sample must be stored to develop changes before the analysis. Furthermore, samples that consist of two or more constituents may result in complicated DSC scans.

Another thermal technique, with different characteristics from those of the DSC instrument,

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Correspondence: M. Angberg, Department of Pharmaceutics, Uppsala Biomedical Centre, Box 580, S-751 23 Uppsala, Sweden.

is high-sensitivity isothermal heat-conduction microcalorimetry. It is an analytical technique which can continuously monitor processes that consume or produce heat. It is non-specific and the interpretation of the output signal can therefore be difficult. However, a signal significantly different from the baseline means that a process is in progress in the sample, although it is not possible to decide directly whether it is destructive. In pharmaceutics, this technique could be a valuable complement to other analytical methods, especially for stability measurements, but also for other types of studies, as exemplified below.

The degradation rate for the hydrolysis of acetylsalicylic acid in aqueous solution at pH 1.1 (Angberg et al., 1988) and at different pH values (Angberg et al., 1990) has been investigated. The latter publication also includes additional methods to evaluate the microcalorimetric response. Other studies concern microemulsions in the absence and presence of drug (Fubini et al., 1988, 1989) and in vitro dissolution testing of oral controlled release preparations in the presence of artificial foodstuffs (Ashby et al., 1989; Buckton et al., 1989). Pharmaceutical stability studies in the solid state are rare, but have been conducted on cephalosporins (Pikal and Dellerman, 1989) and on lovastatin, a drug sensitive to oxidation (Hansen et al., 1989). Investigations on the adsorption of water vapour onto barbiturate powder surfaces for characterizing surface energetics (Buckton and Beezer, 1988) have also been published. The same technique was also used to investigate the effect of comminution on the surface energy of acetylsalicylic acid (Buckton et al., 1988) and the interaction of various types of microcrystalline cellulose and starch with water vapour (Blair et al., 1990).

Lactose is a disaccharide that exists in two optic isomeric forms; α- and β-lactose. In aqueous solution, lactose undergoes mutarotation, which results in an equilibrium at room temperature consisting of 38% α- and 62% β-lactose. In the solid state, α-lactose is most commonly used in the monohydrate form, generally in granulation formulations (Lerk, 1983). Anhydrous lactose powders, with different ratios of α- and β-lactose, are mainly used as direct compression excipients (Batuyios, 1966; Mendell, 1972; Nyqvist and Nicklasson, 1983; Bolhuis et al., 1985; Whiteman and Yarwood, 1988).

Pharmaceutical excipients for dry formulations are usually investigated to show how they interact with water vapour and how important properties may change when stored under humid conditions, e.g., various anhydrous lactose powders show very different sensitivities in their abilities to withstand the incorporation of hydrate water. Hudson and Brown (1908) stated that lactose can be crystallized in two forms, the monohydrate and an anhydrous form named β-anhydrous milk-sugar. An α-anhydrous form can be produced when α-lactose monohydrate is heated to 125 ° C, which results in hygroscopic anhydrous α-lactose. Berlin et al. (1971) reported that anhydrous β-lactose is completely non-hygroscopic below 97% relative humidity (RH). Above 97% RH it quickly sorbs water, wherein β-lactose can mutarotate to α-lactose and subsequently form the crystalline α-lactose monohydrate. They have also described a form of anhydrous α-lactose that changes to α-lactose monohydrate at a very low humidity. Lerk (1983) also reported that β-lactose exists in a non-hygroscopic form only. Hygroscopic anhydrous α-lactose powder can be produced by heating α-lactose monohydrate, mostly in vacuo, to 100–130 ° C. A non-hygroscopic anhydrous (stable) α-lactose may be formed when α-lactose monohydrate is heated above 130 ° C. Ford and Timmins (1989) reported that β-lactose exists as a stable anhydrous form and additionally as a glassy form, which is stable at lower relative humidities but is hygroscopic at higher humidities. Finally, the *Handbook of Pharmaceutical Excipients* (1986) states that anhydrous lactose becomes monohydrate at 70% RH at room temperature, but does not specify what type of anhydrous lactose is referred to. With the information given above, it is difficult to obtain a consistent and complete picture of the stability at humid conditions of anhydrous lactose.

The object of this study was to evaluate the use of heat-conduction microcalorimetry for the characterization of roller-dried anhydrous lactose powder, its sensitivity to water vapour at room temperature and concurrent crystallographic changes. Included in the paper is a discussion of the sample preparation, the evaluation of the microcalorimetric response and the information that can be obtained from microcalorimetric measurements. Water vapour uptake and DSC measurements have also been performed.

Materials and Methods

Materials

Lactose N.F. anhydrous direct tableting 59009, lot number: 8NH01, Sheffield Products, Norwich, NY, U.S.A.; α-lactose monohydrate, De Melkindustrie, Veghel bv. (DMV), Veghel, The Netherlands.

Anhydrous lactose, which has a high β-content, is usually prepared by crystallization from a supersaturated aqueous solution of lactose by heating it above 93 ° C (Lerk, 1983; Olano et al., 1983). The preparation of the anhydrous lactose used in this study included a roller-drying step. The powder produced is a mixture of α- and β-lactose, in proportions of approximately 35% and 65%, respectively (communication from Sheffield Products). The batch used was analysed using gas chromatography (GC) at DMV, The Netherlands. The α- and β-lactose contents were 31% and 69%, respectively.

Methods

Storage conditions at varying relative humidities. Lactose powder samples, 2.20 ± 0.005 g, were spread on trays (10 cm^2), as an approximately 4 mm layer. The trays were then transferred to desiccators, used here as humidity chambers, having specific relative humidities (RH) regulated by saturated salt solutions 94% (KNO_3), 84% (KCl), 81% (KBr), 75% (NaCl), 58% (NaBr) and 33% ($MgCl_2$) RH, respectively (Nyqvist, 1983). The humidity chambers were placed at ambient temperature (20–23 ° C).

The anhydrous lactose samples were stored in the humidity chambers for 1, 3, 5, 14 or 22 days. Additional samples were also stored at 94% RH for 37, 48, 76, 101 and 112 days, and at 58% RH for 93 days. The α-lactose monohydrate samples were stored for 1, 3 or 5 days.

Water vapour uptake during storage. Water vapour uptake was determined gravimetrically. The weight increase after storage up to 22 days, as described above, was then recorded.

Microcalorimetric measurements. After storage in the humidity chamber, the powder sample was quickly poured into a cylindrical stainless steel vessel with a volume of 4.2 ml. The headspace was between 0.7 and 1.2 ml depending on the powder's water vapour uptake. The vessel was closed immediately with a matching lid, lined with a teflon disc.

The microcalorimeter system used, the 2277 Thermal Activity Monitor (TAM) (Thermometric AB, Sweden), has been described elsewhere (Suurkuusk and Wadsö, 1982). In this investigation, the experimental temperature was 25.0 ° C. A static electric calibration of 10, 30 or 100 μW, whichever was most suitable, was performed before each measurement. A sample vessel, prepared as described above, and a reference vessel, filled with 2.2 g of glass beads, were inserted into the calorimeter. The first 30 min were allowed for temperature equilibration at two levels before reaching the measurement position. After this equilibration, the computer's data collecting program was started. This starting time is hereafter referred to as $t = 0$. The heat flow signal (thermal power, dQ/dt, in $\mu W = \mu J/s$) is monitored as a function of time and it is ideally proportional to the rate of the process. Exothermic heat flow signals are given positive values in this paper.

Each measurement lasted for at least 12 h to monitor the appearance of the signal, but the heat flow value after 2 h has normally been used in the evaluation of the results. Five repeated experiments were performed after 1, 3 and 5 days of storage except for samples stored at 33% RH, for which only two experiments were made. For samples stored 14 and 22 days at all humidities and up to 112 days at 94% RH, single experiments were performed.

Differential scanning calorimetry measurements. A differential scanning calorimeter, DSC 20 (Mettler, Switzerland), was used to characterize the untreated material and the crystallographic changes that appeared as a function of increasing relative humidity and storage time, with special emphasis on the change in hydrate content. The samples were stored in humidity chambers as described above. The DSC sample weights were between 2.5 and 3.5 mg and open aluminium pans were used. The samples were in an atmosphere of nitrogen during the measurements. The heating rate was 10 °C/min and the temperature range was 60–280 °C. Indium was used as a standard (melting point: 156.6 °C, heat of fusion: 28.45 J/g). The integration intervals were between 90 and 155 °C or 90 and 160 °C, to obtain the heat of dehydration. The peak temperatures, i.e., the temperatures corresponding to the apices of the various peaks, were also noted. At least three DSC measurements were made for each combination of relative humidity and storage time, except at 33% RH. Measurements were also performed on untreated α-lactose monohydrate.

Results and Discussion

Water vapour uptake during storage

In Fig. 1, the water vapour uptake in per cent by weight for the different relative humidities (RH) is shown as a function of storage time in the humidity chamber. As the powder was loosely packed in the trays, it was assumed that the water vapour could freely penetrate the entire powder bed. Storage at the highest relative humidity, 94%, gave the greatest weight increase. At 75, 81 and 84% RH, intermediate levels were recorded. At 58% RH, the weight increase was low. A constant level was not reached in 22 days, and this was interpreted as being mainly due to a continuous incorporation of hydrate water in the anhydrous lactose structure. No change in weight was seen at 33% RH, which showed that water was neither adsorbed by nor desorbed from the powder at that humidity.

Microcalorimetry

Evaluation of primary data. Examples of the microcalorimetric response curves over 15 h are shown in Fig. 2. In general, four features are seen. Firstly, the heat flow signals are exothermic. Secondly, the level of the heat flow signals increases as the relative humidity during storage increases: 94% RH showed the highest signals; storage at 75, 81 and 84% RH resulted in intermediate levels and at 58% RH, very low signals were seen. Samples stored at 33% RH showed no signals. Thirdly, the level of the heat flow signals decreases as the storage time in the humidity chambers increases. Fourthly, the heat flow signals decline as the experiment proceeds in the calorimeter.

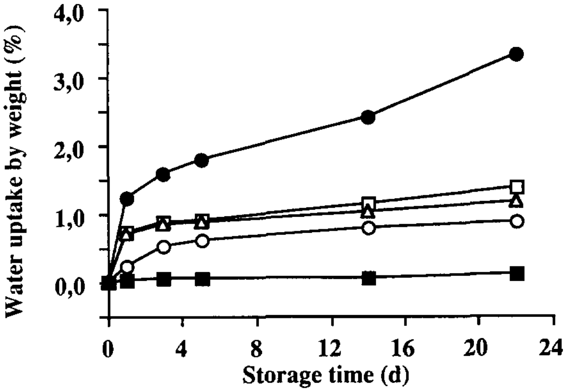

Fig. 1. Water uptake by weight in % as a function of storage time in the humidity chamber at 58 (■), 75 (○), 81 (△), 84 (□) and 94 (●) % RH.

The heat flow signals monitored were interpreted as arising from the incorporation of hydrate water into the anhydrous lactose structure to form α-lactose monohydrate. This process, which most likely involves hydrogen bonding between water and lactose, is exothermic, as discussed by Berlin et al. (1971). The result is consistent with the water vapour uptake shown above and the interpretation was further supported by the fact that there was only a small signal (less than 2 μW) for α-lactose monohydrate powder after storage for 1 day at 94% RH.

The heat flow value obtained after 2 h measurement in the microcalorimeter has been used to characterize the response. However, a heat flow signal which exactly monitors the reaction rate in the humidity chamber would be more desirable. This is not possible, because of the transfer of the samples to the microcalorimetric sample vessel (see below), the time used for temperature equilibration in the calorimeter and disturbances sometimes visible at the beginning of

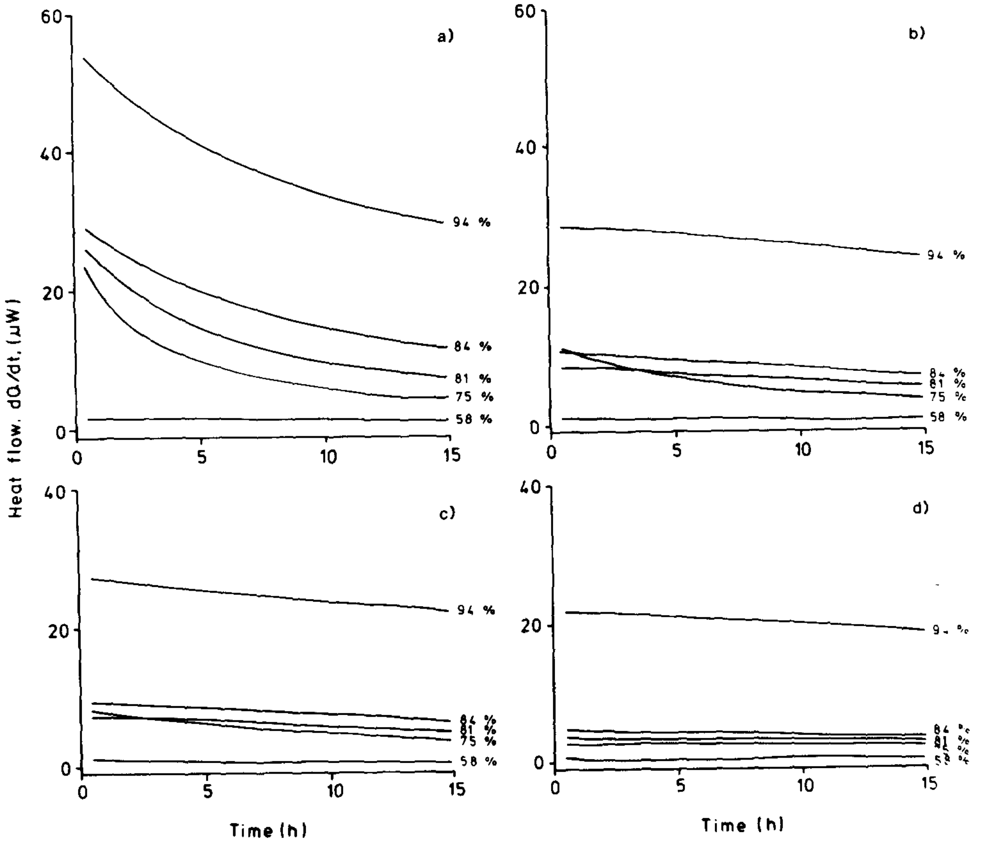

Fig. 2. Primary microcalorimetric heat flow–time curves for 2.2 g anhydrous lactose after storage at 58–94% RH for a, 1 day; b, 3 days; c, 5 days; and d, 22 days.

the measurement. A similar approach has been used to characterize the rate of hydrolysis of acetylsalicylic acid in aqueous solution with acceptable results. The heat flow value after 2 h was then used as a measure for the rate of the process (Angberg et al., 1990).

When the powder was transferred from the humidity chamber to the sample vessel, it was exposed to ambient atmosphere. This influenced the result, as demonstrated by some initial measurements. Three powder samples were stored together in one humidity chamber. The time to transfer powder to the first sample vessel was 15–25 s from the opening of the chamber until the vessel was closed. For the second sample it took an additional 30–40 s, and for the third sample another 30–40 s, to transfer the powders to their respective sample vessels. As can be seen in Fig. 3, the heat flow level (after 2 h) usually

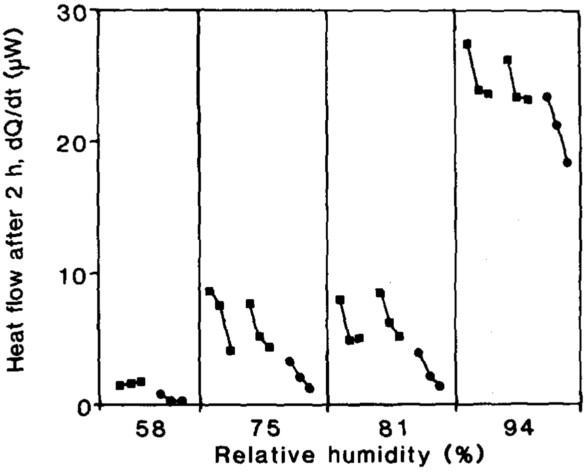

Fig. 3. Heat flow levels after 2 h for 3 samples after storage for 5 days (■) and 14 days (●) in the same humidity chamber at 58, 75, 81 and 94% RH, respectively. The samples were taken from the humidity chamber in the order left to right. See text for further information.

decreases for the second and third sample, which is probably because water disappears from the powder surface. Therefore, only the value of the first sample was used and, in the latter part of the study, only one sample was taken from each humidity chamber. It is obvious that these samples also suffered from the drawback that some adsorbed water disappeared before the vessels were closed.

Changes as a function of storage time. As the storage time in the humidity chambers increases, a decrease in the heat flow levels is seen (Fig. 2). This is also shown in Fig. 4, where the heat flow value (after 2 h) is plotted as a function of storage time. After 1 day of storage, the heat flow signals are at the highest level. The largest difference is between days 1 and 3 and after that the decrease is not as pronounced. There is a large difference in heat flow levels between 94% and the intermediate group: 84, 81 and 75% RH. For powders stored at 58% RH the signals are very low, but they still decrease with time, as for the higher humidities.

The appearance of Fig. 4 is explained by the different response of anhydrous α-lactose and β-lactose to water vapour. Anhydrous α-lactose can incorporate hydrate water directly to become α-lactose monohydrate. β-Lactose cannot incorporate water because of its compact structure, and instead it undergoes mutarotation to become α-lactose at very high humidities, with subsequent incorporation of water (Berlin et al., 1971). The amount of anhydrous α-lactose is largest at the beginning, resulting in the highest rate of incorporation and thus the highest heat flow signal for each humidity level. As the storage time increases, the amount of anhydrous α-lactose decreases and this form disappears eventually. This ought to occur first for powders stored at 94% RH, but as can be seen in Fig. 4, the 94% RH heat flow values are on a much higher level than the other humidities during the whole investigated period. This is the result of the continuous incorporation of water in former β-lactose crystals, which have mutarotated to α-lactose. However, this high heat flow level also decreases as the amount of β-lactose left decreases.

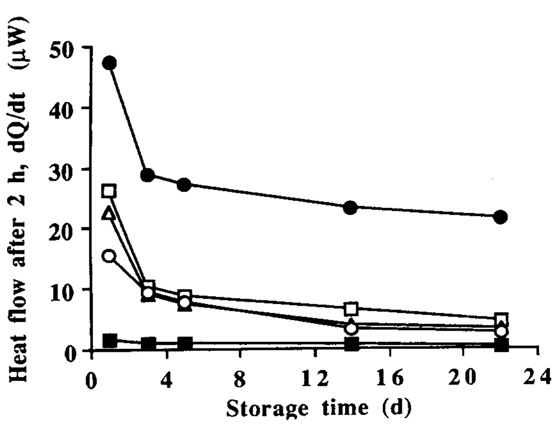

Fig. 4. Heat flow values after 2 h as a function of storage time in the humidity chamber at 58 (■), 75 (○), 81 (△), 84 (□) and 94 (●) % RH. 1, 3 and 5 days are mean values ($n = 5$).

For the intermediate group, 75, 81 and 84% RH, the signals also drop. It is unclear from the microcalorimetric result solely, whether any β-lactose mutarotates at these humidities or whether the decrease in the signals only occurs as the amount of original anhydrous α-lactose decreases. At 58% RH, there are low, but significant, signals which show that it is possible to detect the process even at this humidity using the microcalorimetric technique. As mentioned earlier, the *Handbook of Pharmaceutical Excipients* (1986) reports that anhydrous lactose becomes monohydrate at 70% RH.

The relative standard deviation (S%) for the mean heat flow values after 2 h ($n = 5$) after 1, 3 and 5 days ranges from 3.3 to 18.6% for the four highest humidities. At 58% RH the S% is higher, around 30–40%, due to the low mean value combined with larger relative background disturbances from the instrument. The loss of adsorbed water, during the sample's transfer from the humidity chamber to the vessel (Fig. 3), is considered to be an important reason for the variability.

Changes as a function of the microcalorimetric measurement time. As can be seen in Fig. 2, the heat flow signals decline continuously as a function of time during the measurement in the microcalorimeter. It can be concluded that both water and anhydrous α-lactose, original or transformed from β-lactose, must be available for the incorporation reaction. From being in the humidity chamber with a constant humidity level, where the water consumed in the incorporation process is directly compensated for, the measurement proceeds in the closed calorimetric vessel with only a limited amount of water present, i.e., the amount of non-hydrate water that was transferred with the powder from the humidity chamber. The water subsequently consumed could then obviously not be replaced by water from the environment and therefore the decline of the signal occurs mainly because the concentration of one of the reactants, water, decreases. However, after 1 day of storage in the humidity chamber (Fig. 2a), the decline of the signals is at a maximum. Here it cannot be excluded that the decreasing amount of original anhydrous α-lactose is also of importance in the decline of the signal.

After longer storage times in the humidity chambers, the incorporation reaction proceeds more slowly and the decline during the microcalorimetric measurement is not as rapid as after 1 day of storage (Fig. 2b–d). However, after 3 and 5 days of storage at 75% RH, the heat flow signal still has a pronounced decline, in comparison to measurements made after storage at 81 and 84% RH. This may be because powders stored at 75% RH take up water less rapidly, than at the higher relative humidities investigated as shown in Fig. 1. As the water is adsorbed, it reacts rapidly with the relatively large amount of anhydrous α-lactose left, which gives a pronounced decline in the microcalorimetric signal after 3 and 5 days of storage.

In Fig. 5, heat flow signals after a measurement time of 7 days in the calorimeter are shown. The measurements were performed after storage in the humidity chamber for 1 and 5 days at 94 and 75% RH, respectively. The signal is zero after 7 days at 75%, but there is still a small signal (1.2 μW) after 7 days at 94% RH. The result indicates that the incorporation reaction ceases after a few days in a closed container.

Differential scanning calorimetry

Evaluation of primary data. Fig. 6 represents DSC scans for untreated anhydrous lactose and powder stored for 22 days at 94% RH. For untreated powder (a in Fig. 6), there is a small deviation from the baseline for the release of hydrate water of 15 J/g ($n = 8$) at about 132 ° C, which shows that the material was partially hydrated when the investigation started. A tiny inflection at 218 ° C, a large peak at 235 ° C and a broad peak partly behind the peak at 235 ° C, can also be seen. After 22 days at 94% RH (b in Fig. 6), a much larger peak of dehydration at a peak temperature of 137 ° C can be seen. A growth of the peak at 218 ° C and a reduction of the peak at 235 ° C can also be seen.

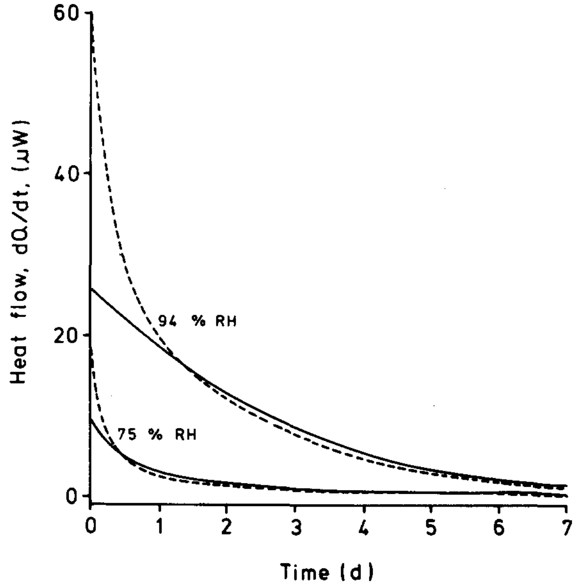

Fig. 5. Heat flow signals as a function of microcalorimetric measurement time. The powders were stored for 1 day (dashed line) and 5 days (solid line) before measurement at 75 and 94% RH, respectively.

Other studies report various melting temperatures for different forms of lactose. For instance, Lerk (1983) reported a melting point of 212 ° C for α-lactose monohydrate, 216 ° C for (stable) anhydrous α-lactose and a melting point for β-lactose of 239 ° C. The β-lactose starting material in that study was analysed by X-ray powder diffraction and was shown to consist of approximately 10% α-lactose and 90% β-lactose. However, the DSC scan for that material only showed one peak at 239 ° C. This is consistent with the DSC scan in Fig 6 (curve a) which also shows only one large peak of fusion. Additionally, Lerk (1983)

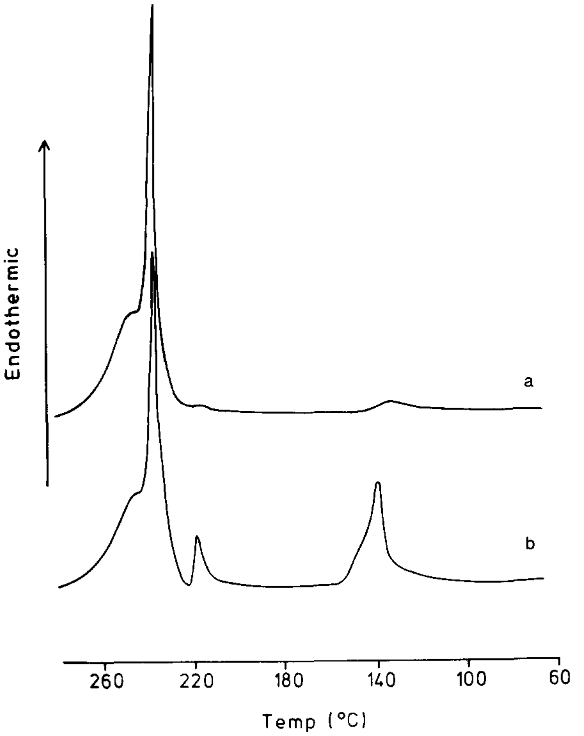

Fig. 6. DSC scans for anhydrous lactose (a) untreated, and (b) stored for 22 days at 94% RH. In the figure, the peak for the dehydration of water at 132 ° C (a) and 137 ° C (b), the melting peaks for α-lactose at 218 ° C and for β-lactose at 235 ° C, are seen. The broad peak at the end of the investigated temperature interval probably represents charring and decomposition.

followed the change in β-lactose content during heating for different forms of lactose powders. The result showed that some of the α-lactose mutarotated to β-lactose before melting, e.g., a powder that consisted of 82% β-lactose at start, had a β-lactose content of 91% when the temperature had increased to 220 ° C. Also Olano et al. (1983) referred to studies where α-lactose mutarotated to β-lactose during heating. This explains why the α-lactose fraction is nearly invisible in the DSC scan (Fig. 6, curve a) for untreated anhydrous lactose used in this study, that consists of 31% α- and 69% β-lactose. This means that the size of the melting peaks shown in the DSC scan for anhydrous lactose, cannot be used to calculate the exact ratio of α- and β-lactose in the sample. However, the peak at 218 ° C, that represents melting of α-lactose, increased in size with storage time in this study, especially at 94% RH. Also powders stored at lower relative humidities, such as at 84% RH for 22 days, show a growth, however minute, of the peak at 218 ° C. At high temperatures, the lactose chars and decomposes (Berlin et al., 1971). The broad peak seen at the end of the investigated temperature interval probably corresponds to this charring and decomposition.

Changes of the dehydration peak as a function of storage time. In Fig. 7, the heat of dehydration obtained from the integrated peak is shown as a function of storage time in the humidity chamber. The heat of dehydration has a positive value, since it is an endothermic heat. The DSC data demonstrate that an increase in both storage time and storage humidity results in an increased amount of hydrate water formed over the investigated period of 22 days, with the exception of samples stored at 58% RH. To confirm that an incorporation process also proceeds at 58% RH, a sample was stored for 93 days. The heat of dehydration showed an increase of 6 J/g, which supports the microcalorimetric result shown earlier. The increase is not as rapid at the beginning at 75% RH, as was seen at 81 and 84% RH, and after 5 days the increase levels off. At 81 and 84% RH, the increase levels off after only 1 day. At 94%, the hydrate increase is greatest at the beginning but continues steadily to increase as a

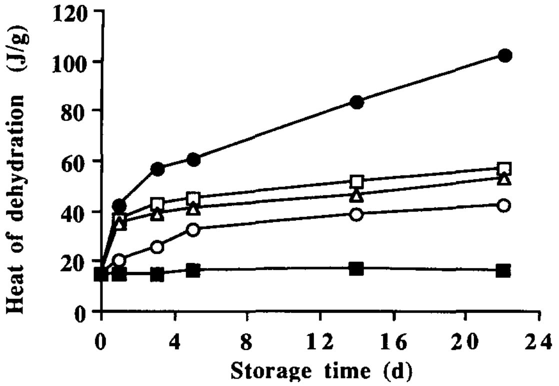

Fig. 7. Heat of dehydration from DSC measurements as a function of storage time in the humidity chamber at 58 (■), 75 (○), 81 (△), 84 (□) and 94 (●) % RH. The values are mean values from at least three measurements.

function of storage time. This must be due to the incorporation of water in mutarotated β-lactose crystals, which is consistent with the microcalorimetric result.

As can be seen in Fig. 7, the values for the heat of dehydration are similar for the middle group, especially for the powders stored at 81 and 84% RH, but also after 5 days for powders stored at 75% RH. This is one important reason why the mean heat flow values shown in Fig. 4 are not always significantly different from each other at these humidities.

In Fig. 8, the heat of dehydration, endothermic (by DSC), and the heat flow level, exothermic (by microcalorimetry), are shown as a function of storage time in the humidity chamber at 94% RH for up to 112 days. The DSC data show a plateau between 76 and 112 days of storage at 181 ± 3 J/g (95% confidence interval). Furthermore, the β-lactose peak at 235 ° C in the DSC scan is no longer visible after 76 days, because it disappears behind the peak corresponding to charring. Then only the α-lactose peak at 218 ° C and the dehydration peak are seen. The DSC data indicate very strongly that the incorporation of water is completed. However, the microcalorimetric heat flow after 112 days still results in a signal of about 6 μW. The microcalorimetric technique can therefore be used to indicate processes that are not detectable by DSC. To confirm this statement, a sample that had been stored for 101 days at 94% RH was shown to contain 2.2% β-lactose when analysed by GC. This shows the sensitivity of the microcalorimetric system in comparison to standard DSC instruments.

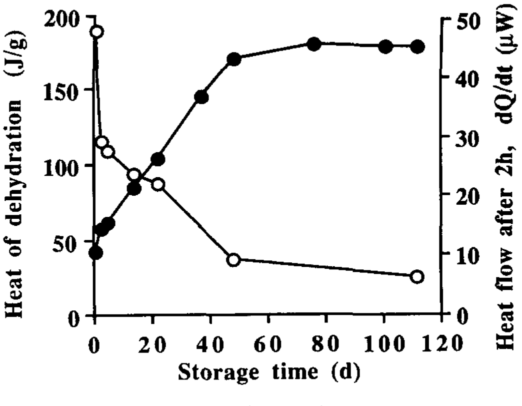

Fig. 8. Heat of dehydration (DSC, ●) and heat flow after 2 h (microcalorimetry, ○) as a function of storage time at 94% RH.

The mean value for the heat of dehydration from the plateau, 181 J/g, in Fig. 8, is higher than was obtained for commercial α-lactose monohydrate by 24 J/g, but the peak temperature, 138 ° C, is lower by 9 ° C. This could be explained by different physico-chemical properties for the type of α-lactose monohydrate formed during storage in high humidities in this study and the commercially available type. This was also seen in the DSC scans as they show dissimilar appearances.

The plateau value was used to calculate the monohydrate content in the untreated starting material. The heat of dehydration at the beginning (15 J/g) was divided by the plateau value (181 J/g), which gave a value of about 8% monohydrate.

The incorporation process

Berlin et al. (1971) gave an explanation as to why β-lactose does not exist as a hydrate. β-Lactose has a higher density value than α-lactose so that its structure must therefore be more compact than that of α-lactose. As the energy expenditure is too large to allow an expansion, β-lactose cannot form a hydrate. Instead water vapour is sorbed rapidly above 97% RH, to form a concentrated solution wherein β-lactose undergoes mutarotation to α-lactose and subsequently water is incorporated to form the crystalline α-lactose monohydrate. That investigation was performed at 24 ° C.

In this study, according to both the microcalorimetric and the DSC results, it seems clear that β-lactose mutarotates at 94% RH at room temperature. The mutarotation also proceeds at lower relative humidities than 94% RH, as discussed below. However, the obtained results may only be valid for a powder with a substantial proportion of anhydrous α-lactose originally, which may influence the mutarotation process, and not for a powder consisting solely of β-lactose. As it is assumed that the incorporation of water at the beginning proceeds primarily in the original anhydrous α-lactose present, it means that the water must have easy access to the α-lactose fraction. This situation prevails if the water can penetrate freely through the lactose structure.

To prove that the mutarotation proceeds below 97% RH, the following calculation was made. If a larger heat of dehydration is measured experimentally than can be accounted for, based on the knowledge of the amount of original α-lactose in the starting material (31%), it will demonstrate that β-lactose has been transformed via mutarotation to α-lactose with subsequent incorporation of water. The heat of dehydration, if all original α-lactose is changed, ought to be 56 J/g (31% × 181 J/g). After 3 days, that level has been reached for powders stored at 94% RH. Similarly, after 22 days, that level has been reached for 84%, and is close for 81% RH (Fig. 7). This shows that the mutarotation proceeds at lower relative humidities than just 97% RH.

The change in β-lactose content during storage was used to directly characterize the mutarotation. A sample stored for 22 days at 75% RH was analysed by GC, which showed a decrease in the β-lactose content of 3%. After 22 days at 75% RH (Fig. 7), the heat of dehydration has a value of only 43 J/g. These data demonstrate that the mutarotation proceeds at much lower humidities than 97% RH, and also that it is a parallel process that starts before all the original anhydrous α-lactose has been consumed. The explanation might be that there are loci in the powder with high water activity where this mutarotation proceeds, even if the overall humidity can be regarded as low. As reviewed by Ahlneck and Zografi (1990), reactions may proceed at low total water content for several reasons, e.g., molecular disorder in the crystal lattice so that it may be able to take up more water than the rest of the crystal. An important factor can be the presence of anhydrous α-lactose in the starting material. This form has greater affinity for water and increases the water activity in the powder. An additional explanation for a catalytic effect of the α-lactose could be that as water is incorporated into anhydrous α-lactose, neighbouring β-lactose molecules may be activated and undergo mutarotation with greater ease.

The mutarotation of β- to α-lactose is an endothermic process. The heat that is necessary to achieve the mutarotation (Hudson and Brown, 1908) is small in comparison to the heat released in the incorporation process. This means that the mutarotation gives only a small reduction of the total heat flow measured in the microcalorimeter. The decrease in heat flow in Fig. 4 is therefore almost entirely a result of the slower incorporation of water into decreasing amounts of lactose.

It may be of interest to investigate the actual rate-limiting steps for the incorporation reaction. The figures for the water vapour uptake (Fig. 1) and the heat of dehydration values (Fig. 7) have a strong resemblance, which shows that the water vapour taken up goes mainly into the hydrate form. This indicates that the availability of water is the rate-limiting step, i.e., storage at a high relative humidity gives a high incorporation rate. This is especially seen in Fig. 2a, which shows the importance of the humidity level.

The curves in Figs 1 and 7 and the microcalorimetric heat flow curves in Fig. 4 have biphasic appearances, except for powders stored at 58% RH. The first phase represents primarily the incorporation into original anhydrous α-lactose. Here, the relative humidity (the water activity) is the rate-limiting step, as indicated above. The second phase represents, at least for powders stored at 94% RH, primarily the incorporation into former β-lactose molecules that have mutarotated to α-lactose. The mutarotation of β-lactose therefore becomes the rate-limiting reaction, yielding the second phase of the curve. This is also shown in Fig. 8. For samples stored at 94% RH, it takes only 3 days to reach a hydrate level of 31%, which probably includes most of the original α-lactose, but an additional 73 days to change approximately all the β-lactose to α-lactose monohydrate. For the humidities that do not allow the β-lactose to mutarotate at an extensive rate (75, 81 and 84% RH), the second phase represents a combination of hydration of the small amounts of original anhydrous α-lactose left and α-lactose obtained from mutarotated β-lactose molecules.

Conclusions

Using the microcalorimetric technique, it has been possible to observe the heat flow from the incorporation of hydrate water in roller-dried anhydrous lactose after just 1 day of storage at a relative humidity as low as 58%. After longer storage times at the highest humidity investigated, 94% RH, the microcalorimetric technique could still monitor processes that were not detectable with a standard DSC instrument. The study has been conducted at ambient temperatures, often desirable when stability studies are performed.

The incorporated process is biphasic, determined by two rate-limiting factors, i.e., the availability of water (phase 1) and the mutarotation of anhydrous β-lactose to α-lactose (phase 2).

For drug substances sensitive to, e.g., hydrolysis, anhydrous lactose with a high β content is useful as a direct compression excipient, since at normal relative humidities it will not take up much water and at high relative humidities the water is probably competitively incorporated into the lactose structure instead of taking part in the hydrolytic reaction.

Microcalorimetry has proved its usefulness in solid state stability work yielding data that disclose subtle and complex processes.

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