International Journal of Pharmaceutics, 83 (1992) 11–23

IJP 02735

Evaluation of heat-conduction microcalorimetry in pharmaceutical stability studies.
VI. Continuous monitoring of the interaction of water vapour with powders and powder mixtures at various relative humidities

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(Received 1 November 1991)
(Accepted 2 December 1991)

Key words: Microcalorimetry; DSC; Anhydrous lactose; Microcrystalline cellulose; Water; Hydration; Stability

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Summary

The water vapour uptake by microcrystalline cellulose (MCC) and a powder mixture composed of roller-dried β-lactose (which consisted of 22% anhydrous α-lactose, 9% α-lactose monohydrate and 69% anhydrous β-lactose) and MCC was continuously monitored at 100% relative humidity (RH) using isothermal heat-conduction microcalorimetry at 25.0°C. For the powder mixture, three phases of water vapour uptake were distinguished, i.e., firstly the absorption of water by MCC, secondly the incorporation of water of hydration into the original anhydrous α-lactose fraction and finally incorporation of water into anhydrous α-lactose that had been obtained by mutarotation from the β-lactose fraction. The combination of the cumulative heat values for the single materials were comparable to the mixture of the two, i.e., the processes were additive and the powders did not interact. However, for mixtures that had been stored for a long time at 94% RH and later monitored at 100% RH, an unknown process was indicated by the heat flow curve. Continuous measurements were also performed at 75, 81 and 94% RH. The interpretation of the heat flow curves for the mixtures was not as straightforward at the lowest humidities investigated as at 100% RH, but the water vapour was absorbed by the MCC first, in these cases also. At both 100 and 94% RH, the incorporation rate of water in roller-dried β-lactose alone was shown to be more rapid than in the mixture with MCC. At 75 and 81% RH, the difference in the extent of the incorporation process was small after 14 days exposure.

Introduction

In a previous study, a novel microcalorimetric measurement technique was designed and evaluated for continuous measurement at 100% RH. The technique involved the presence of a small container filled with water within the sample vessel as a miniature humidity chamber (Angberg et al., 1992). The technique was evaluated by investigating how the incorporation rate of water of hydration differed for various types of anhydrous lactose powder, depending on their respec-

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tive anhydrous α- and β-lactose content. The technique showed several advantages in relation to those in some earlier studies, in which the powders were stored beforehand in humidity chambers (Angberg et al., 1991a,b). The whole incorporation process, except the very initial reaction, could be continuously monitored in an abundant amount of water vapour. The powder sample size was substantially reduced and the transfer of powder from the humidity chamber to the sample vessel at ambient atmosphere was not enforced.

The aim of this study was to further evaluate the technique reported earlier by investigating how a powder mixture interacts with water vapour at 100% RH and to investigate whether the heat flow curves obtained were related to the single components of the mixture or whether other reactions in the binary system were also monitored. It was also of interest to evaluate the technique at lower humidities than 100% RH, because this may be more relevant in pharmaceutical stability studies.

Materials and Methods

Materials

Lactose N.F. anhydrous direct tableting 59009 (prepared by roller-drying, called roller-dried β-lactose), lot no. 8NH01, Sheffield Products, Norwich, NY, U.S.A., Pharmatose® 200M (designated as commercial α-lactose monohydrate), De Melkindustrie Veghel bv (DMV), Veghel, The Netherlands and Avicel® PH 101 (microcrystalline cellulose, referred to throughout as MCC), lot no. 6823, FMC Corp., Philadelphia, PA, U.S.A., were obtained from the indicated sources.

The roller-dried β-lactose used in this study consisted originally of approx. 69% anhydrous β-lactose, 22% anhydrous α-lactose and 9% α-lactose monohydrate. The commercial α-lactose monohydrate consists almost totally of α-lactose monohydrate. The water content in untreated MCC is approx. 4% (information from FMC Corp.).

Methods

Mixing

Equal weights of roller-dried β-lactose and MCC or commercial α-lactose monohydrate and MCC were mixed in a 2 l Turbula mixer (W.A. Bachofen, Switzerland) for 120 min.

Microcalorimetry

Measurements at 100% RH A powder sample was weighed directly into the microcalorimetric sample vessel, a 3.2 ml glass vial. A small container filled with water was placed within the sample vessel. The vessel was closed and 100% RH was eventually attained. Immediately after closure, the sample vessel and a reference vessel were inserted into one of the isothermal heat-conduction microcalorimeters in the 2277 Thermal Activity Monitor (Thermometric AB, Sweden) (Suurkuusk and Wadsö, 1982; Angberg et al., 1988). The vessels were temperature equilibrated for 30 min, before the computer's data collecting program was started ($t = 0$). The experimental temperature was 25.0°C. The heat flow signals (dQ/dt, in μW), which are ideally proportional to the rate of the process, are monitored as a function of time and the cumulative heat is calculated by the computer. Exothermic heat flow signals are given positive values in this paper. A detailed description of the technique has recently been published (Angberg et al., 1992).

Samples of 50.0, 100.0 or 200.0 ± 1.0 mg of the roller-dried β-lactose/MCC mixture, 200.0 ± 1.0 mg of the commercial α-lactose monohydrate/MCC mixture and 100.0 ± 1.0 mg MCC alone, were monitored as described above.

Measurements at 75, 81 and 94% RH Saturated salt solutions were used to achieve relative humidities lower than 100% RH. The salt and water were mixed beforehand at ambient temperature, 20–23°C, which meant that the solutions were saturated at the time of use and that heat obtained from dissolution was avoided. The small container was filled with the appropriate saturated salt solution to obtain 75% RH (NaCl), 81% RH (KBr) and 94% RH (KNO_3), respectively (Nyqvist, 1983), but otherwise the technique was the same as at 100% RH. Measurements were then performed with 100.0 ± 1.0 mg roller-dried β-lactose, 100.0 ± 1.0 mg MCC and 200.0 ± 1.0 mg of the roller-dried β-lactose/MCC mixture as described above.

Differential scanning calorimetry (DSC)

DSC measurements were carried out to obtain the heat of dehydration values for the roller-dried β-lactose. A DSC 20 (Mettler, Switzerland) was used as before (Angberg et al., 1991a). The procedure described earlier (Angberg et al., 1991b), used to correct the dehydration value for the roller-dried β-lactose in a mixture with MCC, was evaluated in this study. The DSC sample weights were between 2.6 and 3.6 mg for the lactose samples and between 3.2 and 3.9 mg for the mixed samples. The endothermic heat of dehydration is given positive values in this paper, each taken from the means of at least three measurements.

Water uptake

The water uptake was obtained gravimetrically by accurately weighing (5 decimal balance) the powder samples before and after the microcalorimetric measurement and recording the weight increase as described before (Angberg et al., 1992).

Interrupted measurements at 100% RH

In a series of measurements at 100% RH, the microcalorimetric measurements were terminated before the incorporation of water of hydration was completed, after 2, 4, 6, 8, 12, 18, 24, 48, 72, 96, 120 and 144 h for 200.0 mg of roller-dried β-lactose/MCC samples. The cumulative heat, the water uptake and the heat of dehydration values were determined for the various measurements.

Measurements at 100% RH after storage at 75–94% RH

Samples of 1.5 g roller-dried β-lactose/MCC mixtures were prestored at 75, 81 or 84% RH for 2 months or at 94% RH for 2 or 3 months in humidity chambers as previously described (Angberg et al., 1991b). Thereafter, 200.0 ± 1.0 mg each of these powder mixtures was monitored at 100% RH. For the samples prestored at 94% RH, the heat of dehydration values were determined both before and after the microcalorimetric measurements.

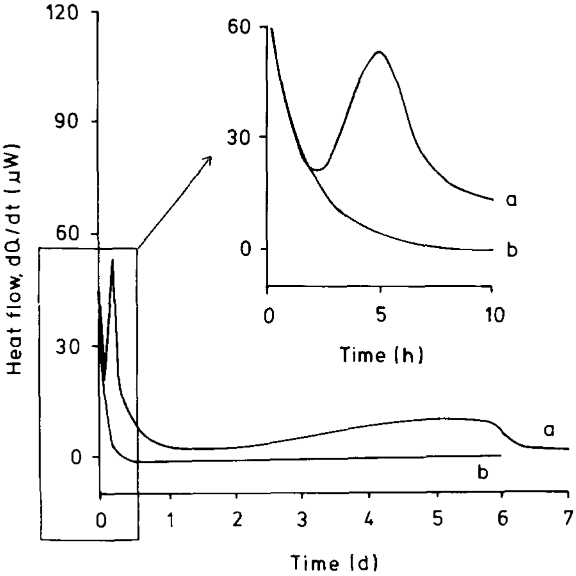

Fig. 1. Heat flow curves for (a) 200.0 mg roller-dried β-lactose/MCC mixture and (b) 100.0 mg MCC, monitored at 100% RH. In the inset the same curves are extended for the first 10 h.

Results and Discussion

Measurements at 100% RH

The appearance of the heat flow curves

In Fig. 1, the heat flow curves for 200.0 mg of the roller-dried β-lactose/MCC mixture (curve a) and for 100.0 mg of MCC alone (curve b) are shown together with an extension of the curves for the first 10 h. The curve (Fig. 1b) for MCC alone shows an exothermic heat flow, which decreases to zero within 8 h. The curve (Fig. 1a) descends for the first 2.5 h, exactly as for the MCC curve and reaches a first minimum, then it ascends drastically, reaches a maximum after 5 h and descends again to a minimum after about 1 day. The curve then increases slowly and after about 6 days falls to near zero.

Previous studies on roller-dried β-lactose

The anhydrous α- and β-lactose contents of the lactose powder investigated are very important for the incorporation rate of water of hydration. α-Lactose can directly accommodate a water molecule in the crystal lattice, but β-lactose, because of its compact structure, must mutarotate to α-lactose before water can be incorporated (Berlin et al., 1971). In a preceding paper (Angberg et al., 1991a), it was shown that the incorporation of water into roller-dried β-lactose is biphasic. The first phase represents incorporation of water into original anhydrous α-lactose, for which the rate-limiting factor is the relative humidity to which the powder is exposed. The second phase represents incorporation into anhydrous α-lactose which has been transformed from mutarotated β-lactose. Here, the mutarotation was shown to be a rate-limiting step. In that study, the powder samples (2.2 g) were stored at 33–94% RH before the microcalorimetric measurements.

In a recent article (Angberg et al., 1992), the same technique was used as in this study at 100% RH. The heat flow curve for a 100.0 mg sample of roller-dried β-lactose clearly showed the biphasic appearance. The initial high heat flow signal rapidly descended, and a minimum value was reached after about 8 h. This first part corresponded mainly to the first phase. After that, the curve increased slowly and after about 4 days measurement, the heat flow decreased to zero. This second part corresponded mainly to the second phase. The mutarotation was also a rate-limiting step at 100% RH. The ascending curve for the second part was due to an increased incorporation rate. When the second part decreased to zero heat flow, the incorporation process was completed.

The interpretation of the heat flow curve for MCC alone

MCC is partly amorphous (Nakai et al., 1977) and as such it is able to absorb much water within the disordered structure (Ahlneck and Zografi, 1990). In an earlier paper (Angberg et al., 1991b), MCC samples (1.0 g) were investigated after storage in humidity chambers at 75–94% RH. The heat flow values were very low, around 1 μW after 1 day of storage. The low values were due to the fact that the absorption of water by the hygroscopic MCC was almost completed during the 1 day storage time.

The technique used in this study also allowed the initial processes to be monitored, except for the first 30 min. Fig. 1b shows a signal that corresponds to heat effects when water is absorbed by MCC, which is an exothermic process due to the formation of hydrogen bonds (Khan and Pilpel, 1987; Blair et al., 1990). However, with the technique used, the vaporization of water is a continuous process, to maintain 100% RH. This is an endothermic process. As the microcalorimeter shows an additive signal, processes with heat flows of opposite sign may cancel each other out. This happened almost directly for the adsorption of water vapour to the crystalline material α-lactose monohydrate (Angberg et al., 1992). The descending exothermic signal for the first 8 h in Fig. 1b corresponds therefore to the heat flow from the absorption of water by MCC, which is larger than the vaporization heat flow. The absorption of water also continues after 8 h, which means that the heat flows are of the same size but opposite sign. However, the signal after 8 h shows a small endothermic signal of 2–3 μW, which reduces to zero over 4 days. This signal may be due to swelling of the MCC, which is an endothermic process involving rupture of some solid-solid bonds (Hollenbeck et al., 1978).

The proposed mechanisms of how water interacts with MCC involve at least a three-stage process, depending on the amount of water taken up (Zografi and Kontny, 1986; Khan and Pilpel, 1987). Zografi and Kontny (1986) suggest a model where (1) water is directly bound to one hydroxyl group per anhydroglucose unit, (2) there is an intermediate stage involving water not directly bound to the primary sites but still influenced by the anhydroglucose units and (3) water is bound to other water molecules as in bulk water. Khan and Pilpel (1987) describe a somewhat different model. In the microcalorimetric study by Blair et al. (1990), a three-stage process is further supported. With the technique used in that study, the water vapour was allowed in from the outside to interact with the powder in the microcalorimeter. In this study, the three-stage mechanism cannot be verified, but the zero heat flow after 8 h may well correspond to stage 3, i.e., water binding to other water molecules (bulk water). Because the MCC was used as received and since the first 30 min was not monitored, it is probably an intermediate stage that is represented during the first 8 h in Fig. 1b.

The interpretation of the heat flow curve for the mixture

The roller-dried β-lactose/MCC mixture was also investigated previously (Angberg et al., 1991b). The samples (1.5 g) were stored at 33–94% RH before the microcalorimetric measurements. The result after 1 day of storage (the first measurement point) was difficult to interpret, because the heat flow levels were similar for 75, 81, 84 and 94% RH and were also much larger than for roller-dried β-lactose alone for 75, 81 and 84% RH, and somewhat lower for 94% RH, when the values had been recalculated considering the lactose content. However, the DSC measurements showed that the incorporation rate was lower for the mixed samples for all the investigated humidities. The suggested explanation involves the distribution of water within the mixture. The water is preferentially taken up by the MCC fraction, as this is the most hygroscopic powder. The consequence of this is that the incorporation rate of water of hydration in the original anhydrous α-lactose is slow at the beginning, but after a while it increases and reaches a maximum rate and decreases again as the amount of the original anhydrous α-lactose decreases. When most of the original α-lactose has changed to the monohydrate the incorporation proceeds in the anhydrous α-lactose that has mutarotated from β-lactose. The time to reach the highest incorporation rate, and the rate in itself, must differ depending on the experimental relative humidity, which made the microcalorimetric result difficult to interpret.

The explanation above describes the appearance of the heat flow curve of Fig. 1a well. At 100% RH, the distribution of water can almost be visualized. The first decline corresponds to absorption by MCC. The peak represents mainly the incorporation into original anhydrous α-lactose and the low signal that increases slowly represents mainly the incorporation into α-lactose transformed from mutarotated β-lactose. This demonstrates the possibility of using this measurement technique to obtain indications of the distribution of water in a powder mixture.

The effect of the equilibration period for the microcalorimetric result

One advantage demonstrated for the technique used in this study is that the initial part of the process can be monitored, except for the first 30 min. This time, which was used for temperature equilibration, may however, be long enough to lose important events. For a 200.0 mg sample of roller-dried β-lactose/MCC, the absorption of water by MCC was readily observed, but when a sample of 100.0 mg of the mixture was monitored, that part had almost been passed within 30 min. For a 50.0 mg sample, the curve started directly with the peak corresponding to incorporation of water into the original anhydrous α-lactose, totally missing the absorption by MCC. This means that a larger sample weight can avoid this problem. The possibility of using a shorter equilibration time has not been evaluated in this study.

The role of MCC in delaying the incorporation process

The superimposed heat flow signals at the beginning of the curves in Fig. 1 indicate the good penetration capability of water vapour in the powder bed. This was further investigated by monitoring a 200.0 mg mixture of commercial α-lactose monohydrate/MCC, which gave an almost identical heat flow curve to that of Fig. 1b. This shows that the curve for MCC alone can account for the heat flow contribution to the roller-dried β-lactose/MCC curve. In Fig. 2a, values from the curves shown in Fig. 1 are plotted for the first 480 min (8 h). The values from the MCC curve were then subtracted from those for

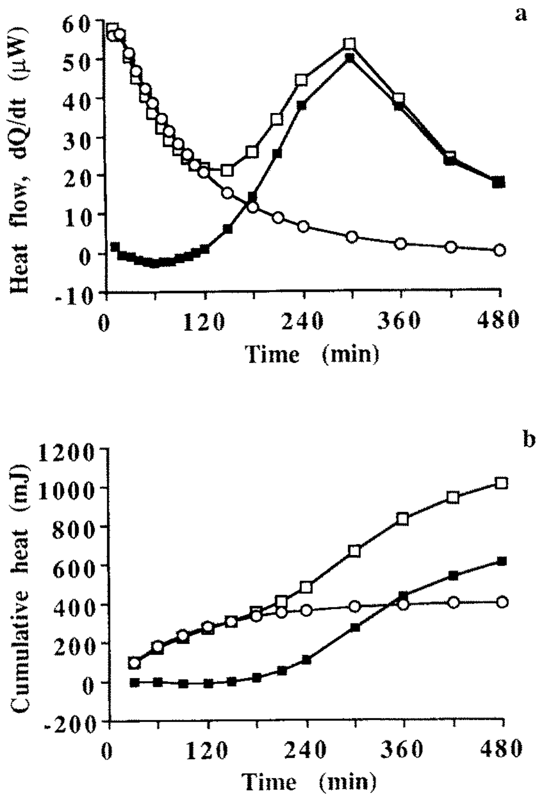

Fig. 2. (a) Values from the heat flow curves presented in Fig. 1, where (□) denotes roller-dried β-lactose/MCC mixture and (○) refers to MCC alone. (■) Represents values obtained by subtraction of the MCC values from those for the mixture, which correspond to the incorporation of water of hydration in lactose. (b) The calculated cumulative heat for the curves presented in (a).

the mixture to obtain a constructed heat flow curve which corresponds to the incorporation of water into roller-dried β-lactose alone. There is a delay of approx. 120 min (2 h) before the incorporation into the original anhydrous α-lactose starts. In Fig. 2b, the cumulative heat values are displayed. Firstly, there is no heat evolved from the incorporation process at the beginning and, secondly, the cumulative heat for the absorption of water by MCC ends at approx. 400 mJ. However, this heat value cannot be used to calculate the extent of the absorption of water in the intermediate stage, discussed above. This is due not only to the fact that the endothermic heat of vaporization is included in the heat monitored, but also to that both formation and breaking of bonds occur. This also reduced the expected enthalpy change in the microcalorimetric study performed by Blair et al. (1990).

The additivity of the processes

The result in Fig. 2 indicates that the heat flow from the various processes can be directly related to the components in the mixture. To further investigate this, the cumulative heat values of the processes are compared. The curve of Fig. 1a, corresponding to the 200.0 mg roller-dried β-lactose/MCC mixture, ended at 4.25 J after 165 h (nearly 7 days). For 100.0 mg roller-dried β-lactose alone, the heat obtained was 3.55 J (Angberg et al., 1992), which gives a discrepancy of 0.7 J. This difference is reduced by 0.4 J due to the absorption of water by MCC. Furthermore, the incorporation process for roller-dried β-lactose alone was not monitored during the temperature equilibration period of 30 min, which would correspond to about 0.15 J (Angberg et al., 1992). This increases the value that can be accounted for to 4.1 J, as there is no incorporation process during the equilibration period in this study. The last 0.15 J can probably be explained by the fact that the curve (Fig. 1a) does not end at a zero heat flow after 165 h, but continues as a plateau that begins after 152 h, and it is probably here that the incorporation process is completed. The cumulative heat after 152 h is 4.2 J, which corresponds closely to that expected from the two separate powders. The heat obtained from the absorption by MCC and the incorporation of water into the roller-dried β-lactose is therefore shown to be additive in a mixture. This fact indicates that there is no interaction between the powders.

The uptake of water and the duration of the incorporation process

The water taken up by the roller-dried β-lactose/MCC mixture and MCC alone was investigated by interrupting the microcalorimetric measurements and measuring the weight increase for the different time intervals. The weight increase was 27.6 mg after 165 h for the 200.0 g mixture. The amount taken up was larger for the mixture than for the separate 100.0 mg samples of MCC or roller-dried β-lactose. However, if the water uptake is expressed in per cent by weight, the hygroscopic MCC sample shows the largest value, 18.2% w/w, compared to 15.5% for the roller-dried β-lactose and 13.7% w/w for the mixture, all measured after 144 h. This observation will be discussed further below. For the MCC samples, the water uptake was 7.9% w/w after 8 h, which means that there was a substantial uptake after the heat flow had attained an approximately zero value. The values for roller-dried β-lactose alone were taken from the earlier study (Angberg et al., 1992). The weight increased then not only due to the incorporation, but also due to adsorbed water.

In an earlier study, it was shown that it took longer to change all anhydrous lactose to the monohydrate, i.e., to finish the incorporation process for roller-dried β-lactose in a mixture with MCC, than when the roller-dried β-lactose was alone (Angberg et al., 1991b). With this measurement technique the incorporation process also proceeds for a longer time for the mixture. It ends just after 6 days (Fig. 1), compared to 4 days for roller-dried β-lactose alone (Angberg et al., 1992). It was shown in that study that the incorporation rate increased in the second phase, i.e., the phase that included the rate-limiting mutarotation from β- to α-lactose. The suggested explanation for this was that the amount of adsorbed water increased rapidly, the mutarotation process became thereby more facile and both the mutarotation and subsequently the incorporation rate increased. The extended duration of the incorporation process for the mixture is explained by the competitive absorption of water by the MCC fraction. The water taken up is therefore not available to the same extent as a medium for the mutarotation process, as when roller-dried β-lactose alone was investigated.

However, another reason for the prolonged incorporation duration for the mixture is the sample weight. The result for the 200.0 mg mixture of roller-dried β-lactose/MCC has consequently been compared to that for 100.0 mg roller-dried β-lactose alone, to maintain the amount of lactose as the same. But as shown above, the water uptake in per cent by weight is lower for the mixture than for the separate powders alone. So, even if there is a surplus of water present in the sample vessel, the rate of water vaporization influences the incorporation rate, since the water taken up must be shared by a larger sample weight.

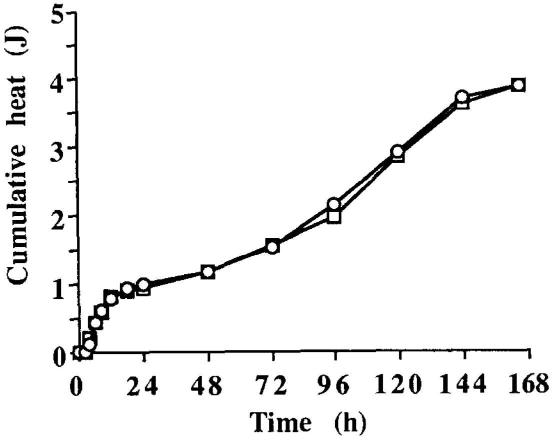

Fig. 3. Calculated cumulative heat for the interrupted measurements of 200.0 mg roller-dried β-lactose/MCC mixtures (□) and at the same time intervals for the continuous measurement presented in Fig. 1 (○). The heat that corresponds to the absorption by MCC (0.4 J) has been subtracted from all values.

Reproducibility of the used microcalorimetric technique

The measurements for 200.0 mg roller-dried β-lactose/MCC samples were interrupted after various time intervals and the cumulative heat values were calculated and plotted in Fig. 3. The cumulative heat values obtained for the same time intervals for the continuous measurement presented in Fig. 1a, which lasted for 165 h, are also included. As can be seen, the values for the two curves are almost identical, which shows that the technique has good reproducibility at 100% RH.

Evaluation of the DSC measurements

The extent of the water incorporation into roller-dried β-lactose was measured using DSC. The heat of dehydration values are given per g, calculated from the sample weight used for each measurement. Because the sample consisted of a roller-dried β-lactose/MCC mixture, a correc-

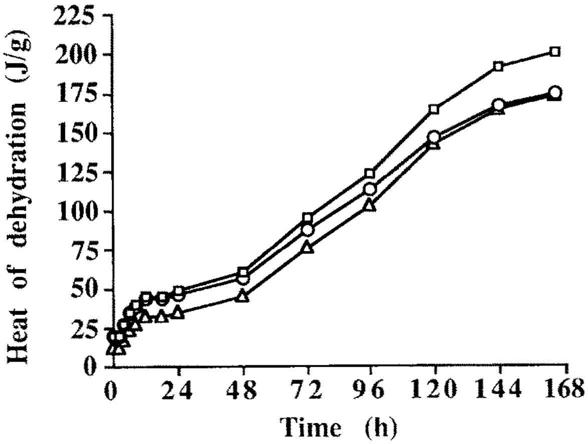

Fig. 4. Heat of dehydration values for 200.0 mg roller-dried β-lactose/MCC mixture after interrupted measurements at 100% RH corrected by different methods to express the values per g lactose. The values (△) have been multiplied by two. The values (○) have been multiplied by two and by a factor from a correction curve that is explained in the text. The values (□) have first been corrected for the water taken up, thereafter multiplied by two and a factor from the correction curve.

tion procedure has previously been developed in order to express the heat of dehydration value per g of lactose (Angberg et al., 1991b). This correction procedure is further evaluated in this study, to allow the most favourable comparison possible with the microcalorimetric result.

In Fig. 4, the increase in the heat of dehydration as a function of time for the interrupted measurements for the roller-dried β-lactose/MCC samples are shown for three extensions of the correction procedure. The last value after 165 h is from the sample used in Fig. 1. The curve at the bottom corresponds to heat of dehydration values that have been multiplied by two, as the sample consisted of 50% roller-dried β-lactose. This correction procedure yields very low values at the beginning. The middle curve corresponds to the values obtained from the lower curve, which have also been multiplied by a factor from a correction curve used also earlier (Angberg et al., 1991b), in order to reduce the influence of the MCC admixture on the integration of the dehydration peak. The curve at the top corresponds to values in which the 'heat of dehydration per g' has been altered to compensate for the weight of water taken up during the same time interval. The new values were multiplied by two and thereafter by a factor from the correction curve.

The best correction procedure must be the one that ends at the same maximum heat of dehydration value as that obtained for roller-dried β-lactose alone, namely, 181 ± 3 J/g (95% confidence interval, $n = 10$) (Angberg et al., 1992). The final values are 175 J/g for the curve at the bottom, 177 J/g for that in the middle and 203 J/g for the curve at the top. The last value shows that correction for the water taken up gives values that are too high. This is due to the fact that the water absorbed at high humidities by the MCC will easily be desorbed when the powder samples are in ambient atmosphere. The DSC samples will therefore not contain as much water as was weighed immediately after the microcalorimetric sample vessel was opened, since the samples were usually in ambient atmosphere for about 1–3 h before the DSC measurements. The final value for the middle curve was 177 J/g, which is close to 181 J/g considering the uncertainty in the estimation of the mean value. Furthermore, that curve also starts at the same heat of dehydration value (17 J/g) as the roller-dried β-lactose alone. For the DSC result presented below, the correction procedure described for the middle curve will therefore be used for the mixed samples.

All the curves in Fig. 4 show a general similarity to the cumulative heat curves plotted in Fig. 3. However, they cannot become totally alike. This is due to the fact that the cumulative heat is reduced by an endothermic heat from the mutarotation process (Angberg et al., 1992).

The effect of prestorage on the microcalorimetric characterization

In the preceding study (Angberg et al., 1992), it was shown that roller-dried β-lactose that had been prestored at lower humidities and afterwards monitored at 100% RH would show a changed, but explainable, heat flow curve if the anhydrous content had decreased during the prestorage. In Fig. 5, the heat flow curves are shown for 200.0 mg mixtures of roller-dried β-

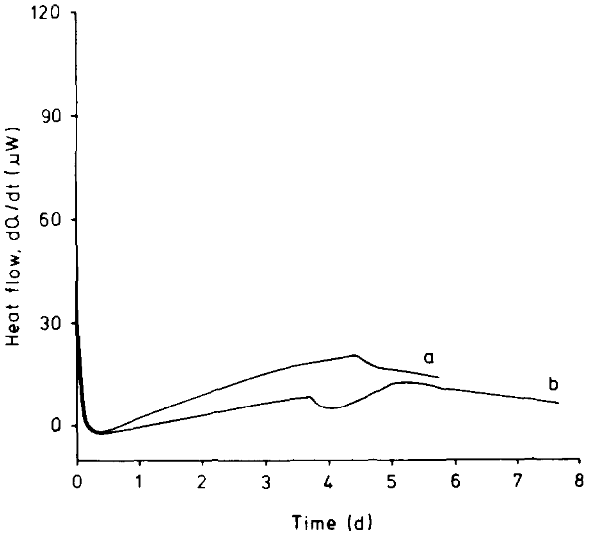

Fig. 5. Heat flow curves for 200 mg roller-dried β-lactose/MCC mixture that had been prestored at 94% RH for (a) 2 and (b) 3 months and thereafter measured at 100% RH.

lactose/MCC that had been prestored in humidity chambers at 94% RH for 2 (curve a) or 3 (curve b) months. They are comparable to that of Fig. 1a for an untreated powder mixture. The curves show both expected and unexpected features. There is no peak corresponding to the incorporation of water into original anhydrous α-lactose, for either curve in Fig. 5, which shows that this process is already completed. However, both curves show the absorption of water vapour by the MCC during the first hours, since the powders had been in ambient atmosphere after the prestorage. This means that much of the water absorbed by the MCC at 94% RH had been desorbed and is now resorbed at 100%. Furthermore, more water is absorbed at 100% RH than at 94% RH. Both curves show a large increase after the absorption heat flow has ended. The curve (Fig. 5a) starts to decrease after 4.5 days, but after 6 days when the measurement was terminated it had not decreased as far as zero heat flow. The same type of measurement was performed twice more and gave the same heat flow curve appearance, which showed that the samples always changed identically. The curve (Fig. 5b) starts to decrease after 3.5 days. After a few hours the curve ascends again and after about 5 days it descends once again. As observed by Angberg et al. (1992), the incorporation reaction ceases earlier for a powder that has been exposed to a high relative humidity for the longer time. This supports the theory that the decrease after 4.5 days (2 months) and 3.5 days (3 months) occurs because the incorporation process actually ends, but that there is another process, which influences the powder mixture.

The changes in the heat of dehydration values were also investigated. The sample prestored for 2 months and used to construct the curve of Fig. 5a started at 105 J/g and ended at 147 J/g. This is lower than 181 J/g, the value for a completed incorporation process. The sample prestored for 3 months and used for plotting Fig. 5b started at 120 J/g, which as expected shows that the incorporation process has come further than for the 2-month sample. A 3-month sample, which had the same appearance as that in Fig. 5b, was terminated after 5 days at a value of 163 J/g, which again shows that the maximum value had not been reached. After 8 days, the sample used for the curve (Fig. 5b) had a value of only 151 J/g, which is even lower than after 5 days.

The explanation of the result above may be that the long storage period at high humidity has decreased the glass transition temperature, T_g, of the powder mixture. An amorphous excipient (in this case the MCC), that has taken up a large quantity of water, may acquire a decreased T_g, because the water works as a plasticizer. If the T_g becomes less than the experimental temperature, the molecular mobility will increase. For a mixture consisting of an amorphous excipient and a crystalline component (in this case the lactose), the water that is located at the (lactose-MCC) interface may plasticize them both and facilitate interactions and phase changes (Ahlneck and Zografi, 1990). This may cause a change in the structure of the lactose, so that the hydrate content decreases, e.g., the lactose dissolves and/or becomes partly amorphous. The DSC scans are also very much deformed with indications of recrystallization of amorphous structures (Lerk, 1983). The extra heat flow monitored by the microcalorimeter is exothermic. This excludes therefore processes like dissolution and the loss of crystallinity of the lactose, which are both endothermic mechanisms. However, if an endothermic process is proceeding at the same time as an exothermic process of higher absolute value, the heat flow ends up as an exothermic signal. This demonstrates that this microcalorimetric technique can monitor continuous changes in the solid sample which, even if they may be difficult to explain directly, may call for further studies with other analytical techniques.

For the powders prestored for 2 months at 75, 81 and 84% RH, no unexpected processes were indicated. All the heat flow curves started with absorption of water by MCC, continued with a small peak corresponding to incorporation of water into what was left of the original anhydrous α-lactose (almost invisible for 81 and 84% RH), and thereafter continued with the incorporation into α-lactose transformed from mutarotated β-lactose. This second phase ended, as expected, earlier for the prestored samples than for the untreated mixture.

Measurements at 75, 81 and 94% RH

The appearance of the heat-flow curves

The possibility of using lower humidities than 100% RH was also investigated. In Fig. 6a–c, the heat flow curves for 100.0 mg roller-dried β-lactose, 100.0 mg MCC and 200.0 mg of the roller-dried β-lactose/MCC mixture are shown for the first 60 h of measurement at 94, 81 and 75% RH, respectively. These measurements continued in reality for 3 days. The curves in Fig. 6a–c, which correspond to roller-dried β-lactose alone, show that the heat flow value at $t = 0$ increases as a function of the relative humidity, as in the previous study (Angberg et al., 1991a). The signal then decreases with different slopes, presumably depending on the hydration rate of the original anhydrous α-lactose, i.e., the first phase of the incorporation process. At 94% RH, the signal is almost parallel to the baseline after about 15 h. The first period after 15 h corresponds to parallel incorporation processes of both phases, which continues in only the second phase after some time, i.e., incorporation of water into α-lactose that has mutarotated from β-lactose.

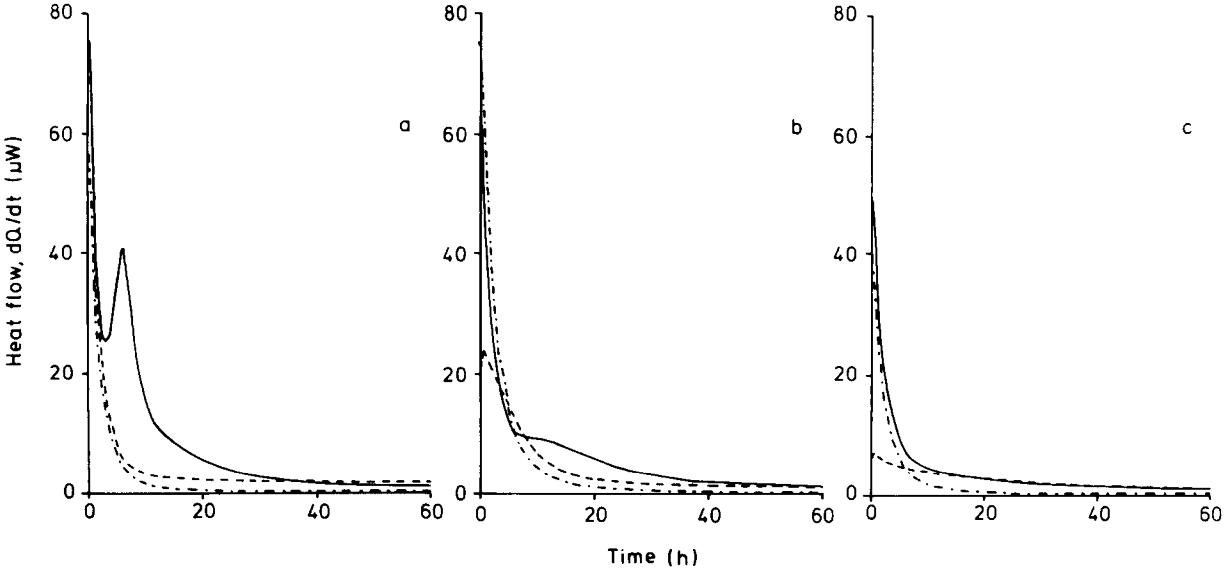

Fig. 6. Heat flow curves for 100.0 mg roller-dried β-lactose (- - - - - -), 100.0 mg MCC (·–·–·) and 200.0 mg roller-dried β-lactose/MCC mixture (———) measured at (a) 94, (b) 81 and (c) 75% RH.

No increased incorporation rate in the second phase was seen for the measurements that lasted 14 days at 94% RH. This is consistent with the result when the powders had been stored in a humidity chamber at 94% RH and measured afterwards (Angberg et al., 1991a), but different from that at 100% RH with the same technique as in this study (Angberg et al., 1992). For the lower humidities, the signal decreases with time and is lower than the heat flow at 94% RH. The mutarotation rate is almost negligible at these humidities (Angberg et al., 1991a).

For the MCC samples, the heat flow curves for the different humidities have similar appearances. The heat flow curves start with high values, which eventually decrease to zero. However, this takes longer than at 100% RH (Fig. 1b).

For the mixed samples, all the heat flow curves start with the decline corresponding to the absorption of water by MCC. This shows again that the water is preferentially taken up by the MCC at the lower humidities also. The heat flow curve at 94% RH resembles the curve at 100% RH (Fig. 1a), but the apex of the peak that corresponds to the incorporation into the original anhydrous α-lactose is lower. The heat flow curve at 81% RH shows a broad, low peak corresponding to this incorporation and the curve at 75% RH does not show any peak, due to the low incorporation rate also seen for roller-dried β-lactose alone.

The additivity of the processes

For the results presented above at 100% RH, it was shown that the combination of the cumulative heat values obtained for the MCC and the roller-dried β-lactose alone were comparable to that obtained for the mixture of the same entities. At lower humidities, this aspect could not be investigated thoroughly, because the longest measurements continued for only 14 days and during this time none of the heat flow curves indicated a completed process, i.e., they had not attained zero heat flow. In Fig. 7a the cumulative heat values for the heat flow curves shown in Fig. 6a–c have been calculated for the 3 days that the measurements lasted. The first bar corresponds to roller-dried β-lactose alone, the second to the mixture and the third to MCC alone. All the bars increase as a function of the relative humidity. The fourth bar at each humidity corresponds to the heat remaining when the cumulative heat for the MCC is subtracted from the heat for the mixture and thus ideally corresponds to the heat from the incorporation process solely. The fourth bar should be somewhat lower than the first, since the incorporation rate is lower in the presence of MCC, shown at 100% RH above and described in a preceding paper (Angberg et al., 1991b). This is the case for all investigated humidities, but the difference at 81% RH seems to be too large. This is due to the large cumulative

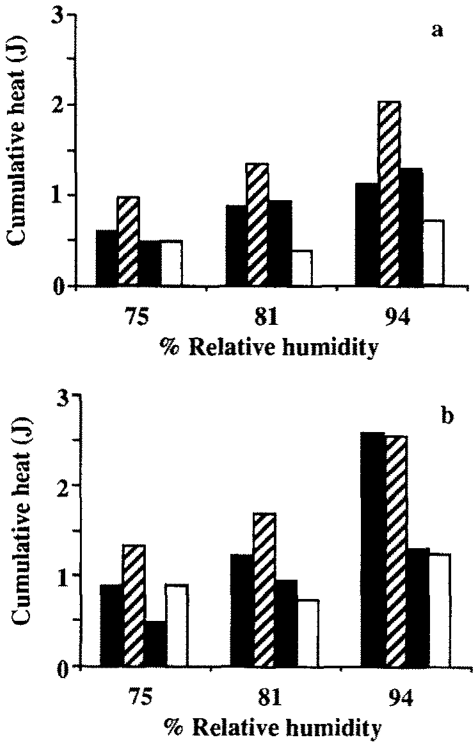

Fig. 7. Cumulative heat values obtained during (a) 3 and (b) 14 days measurement at 75, 81 and 94% RH. Bars: (filled) 100.0 mg roller-dried β-lactose, (diagonally hatched) 200.0 mg roller-dried β-lactose/MCC mixture, (cross-hatched) 100.0 mg MCC, (open) the heat obtained when the MCC values have been subtracted from the values for the mixture. The MCC values for 3 days have also been used for 14 days.

heat obtained for the MCC measurement at 81% RH, which can also be seen in Fig. 6b.

In Fig. 7b, the result after 14 days is shown, except for the fact that the MCC values are taken from Fig. 7a. The bars corresponding to roller-dried β-lactose and to the mixture again increase as a function of the relative humidity. At 75% RH, the difference between the first and fourth bar is negligible. At 81% RH, this difference is large and finally at 94% RH, the difference is very large.

Comparison with DSC measurements

To verify the microcalorimetric result, the heat of dehydration values were measured after the microcalorimetric measurements. The value, 17 J/g, for the untreated roller-dried β-lactose was subtracted from all values to obtain only the increase of the heat of dehydration values, shown in Fig. 8. The first bar in each pair corresponds to the heat of dehydration increase for roller-dried β-lactose alone and the second bar for the roller-dried β-lactose in the mixture. After 3 days (Fig. 8a), the heat of dehydration values at 75 and 94% RH are lower for the mixed samples than for lactose alone, which corresponds well with the microcalorimetric result. At 81% RH, the heat of dehydration value for the mixture is similar to that obtained for roller-dried β-lactose alone, which shows that it really was the large cumulative heat for MCC that reduced the heat corresponding to the incorporation process presented in Fig. 7a.

After 14 days (Fig. 8b), the increase of the heat of dehydration values for the roller-dried β-lactose alone are similar in size at 75% RH, somewhat higher at 81% RH and almost double at 94% RH compared to the corresponding values for lactose in the mixture. The results at 75 and 94% RH are consistent with the result for the cumulative heat. However, the result for 81% RH had again given a falsely low cumulative heat for lactose in the mixture, as it was the same MCC measurement that was used for both 3 and 14 days.

The DSC result at 75% RH shows that the heat of dehydration values are similar after 14 days for the roller-dried β-lactose alone and in the mixture. This might indicate that the incorporation will almost stop after a certain amount of the original anhydrous α-lactose has been consumed. For the first 3 days, the rate is greater for the roller-dried β-lactose alone, but after 14 days the extent of the incorporation into the lactose in the mixture has caught up with the incorporation into the lactose alone and ends approximately at the same level. At 81% RH, the extent of the incorporation is similar after 3 days and somewhat larger for lactose alone after 14 days. At 94% RH, the mutarotation rate is depressed in the mixture, as was the suggested explanation at 100% RH. This results in a large difference be-

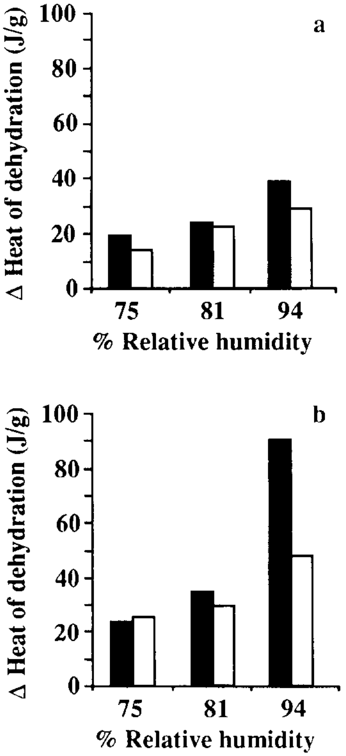

Fig. 8. Heat of dehydration increase obtained after (a) 3 and (b) 14 days at 75, 81 and 94% RH. Bars: (filled) 100.0 mg roller-dried β-lactose, (open) 200.0 mg roller-dried β-lactose/MCC mixture, but recalculated to correspond to the heat of dehydration value per gram of lactose only. The heat of dehydration value for the untreated roller-dried β-lactose was 17 J/g.

tween the incorporation rates. This is also discernible in Fig. 6a, where the heat flow level after 50 h is lower for the mixture than for roller-dried β-lactose alone.

The comparison between the microcalorimetric and the DSC measurements suffers from the fact that the cumulative heat values presented in Fig. 7 do not include the heat for the first 30 min, due to the equilibration period. However, it seems clear that the used technique can also be utilized at lower humidities than 100% RH. The saturated salt solutions will not have any greatly disturbing effects on the heat flow for most of the measurements, but the precision at lower humidities is not as good as at 100% RH.

Conclusions

With the microcalorimetric technique used, the appearance of the heat flow curve for a powder mixture may give indications of the order in which the water vapour interacts with the powder constituents. Furthermore, the interaction between separate powders in a mixture can be investigated. If the cumulative heat values for each separate constituent are added together and found to be the same as for the mixture, they probably do not interact. If, however, the values are not the same as for the mixture, the powders interact or other processes proceed in the sample.

The salt solutions that are used to produce a lower humidity than 100% RH in the sample vessel do usually not disturb the heat flow signal if they are well equilibrated. However, the precision is lower than at 100% RH.

The very initial reaction could not be measured with this technique, because of the necessary temperature equilibration period. Important events may have occurred within this period. This situation can be avoided by using a larger sample weight, which yields an extended duration of the processes.

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