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The use of gravimetric studies to assess the degree of crystallinity of predominantly crystalline powders

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Abstract

An accurate humidity and temperature controlled microbalance system (dynamic vapour sorption) has been used to investigate the water sorption properties of mixtures of amorphous lactose (spray dried) and crystalline α-lactose monohydrate. From the shape of the sorption isotherms it could be seen that the first sorption process for each sample showed higher weight gain due to absorption into the amorphous regions, than was observed for the subsequent adsorption events on the sample. For the first sorption process, the weight fell after the humidity reached about 60% RH, as the amorphous material recrystallised (causing expulsion of absorbed water). On desorption there was always residual water after the first cycle, which correlated reasonably well with the residual that would be predicted if the amorphous material had been converted into the monohydrate form. The water sorption technique can readily be used to demonstrate the existence of amorphous material even for mixtures which contain as little as 0.05% w/w, and it is possible that the residual weight change may be used as an approximate quantification of the original amorphous content of the sample.

Keywords: Lactose; Crystallinity; Amorphous material; Water sorption; Isotherm

1. Introduction

It is well known that processing can influence the functional behaviour of powders. Milling is an example of a process which can alter powder properties (e.g., Florence and Salole, 1976; Buckton et al., 1988; Elamin et al., 1992; Briggner et al., 1994; Saleki-Gerhardt et al., 1994). It is probable that many of the problems associated with batch-to-batch variability of materials relate to changes in the powder surface, induced as a consequence of a change in material production (either accidentally or deliberately). A problem that has persisted is that the analytical techniques which have been used to study powder properties have indicated that various processing stages have little influence, even though differences in performance are noted in products. There have been a number of publications recently which have explored new approaches to the study of small changes in powder crystallinity. Sheridan et al. (1993) have shown that a vapour flow microcalorimetry method was significantly more sensitive than contact angle studies for differentiating between three samples of lactose, which

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were known to behave differently in a product. Saleki-Gerhardt et al. (1994), Briggner et al. (1994), Sebhatu et al. (1994), Ticehurst et al. (1994) and Thompson et al. (1994) have all shown that powder X-ray diffraction is not a sensitive method of studying small changes in powder properties, indeed XRD is unable to detect amorphous material in powder samples unless it amounts to as much as 10% of the total (e.g., Saleki-Gerhardt et al., 1994). It has been shown (e.g., Briggner et al., 1994; Sebhatu et al., 1994; Buckton et al., 1995a) that it is possible to characterise the content of amorphous material in a powder sample by use of isothermal microcalorimetry. The lower limit of resolution is yet to be proven, but appears to be in the order of 0.5–1.0% amorphous content (Briggner et al., 1994; Buckton et al., 1995b). The basis of the microcalorimetry experiment is to seal the powder in a glass ampoule with a tube containing a saturated salt solution. The humidity is then controlled at a level such that the amorphous material in the powder will be plasticised by the water and will then recrystallise in the cell (NB: vapours other than water can be used for water insoluble materials). The area under the power-time curve for the recrystallisation event is then compared to that for an amorphous standard to quantify the degree of crystallinity of any sample. Other approaches to the study of crystallinity include DSC (Otsuka and Kaneniwa, 1990; Saleki-Gerhardt et al., 1994; Sebhatu et al., 1994), infrared spectroscopy (Otsuka and Kaneniwa, 1990) and solution calorimetry (Pikal et al., 1978; Grant and York, 1986; Hendriksen, 1990; Thompson et al., 1994)

In the current study, the process of recrystallisation of lactose has been followed gravimetrically, in order to probe the sensitivity of using a non-calorimetric approach to the investigation of variability in powder samples.

2. Method

Samples of amorphous lactose were prepared by spray drying as described previously (Briggner et al., 1994). Mixtures were prepared containing a known quantity of spray dried lactose and crystalline α-lactose monohydrate. The α-lactose monohydrate was pre-conditioned by raising the material to 85% relative humidity (RH) and then desiccating prior to mixing (this was to ensure that any amorphous content in the 'crystalline' material had been removed prior to study). The powders (approx. 1 g quantities) were mixed in a small glass vial which was wrapped in cotton wool and placed in a larger glass bottle in a Turbula mixer.

The gravimetric studies were undertaken in a humidity controlled microbalance system (dynamic vapour sorption apparatus, Surface Measurement Systems, UK). The construction of the dynamic vapour sorption (DVS) system is based on a Cahn microbalance housed in an incubator to control temperature. Humidity was controlled by flow of dry nitrogen through switching valves, which determine the amount of the total flow to pass through a humidification stage. The apparatus was computer controlled, allowing a pre-programming of the sorption and desorption isotherms. Samples (50 mg) were loaded onto one side of the twin pan balance, and the programme set to control the humidity at 0% for 1 h, then to raise the humidity in steps of 10% RH, in each case the programme allows either a time to be set for holding at each humidity, or to advance to the next humidity after equilibrium had been reached (judged by setting a desired rate of change of mass with time, dm/dt). In the experiments reported here the programme was set to advance the humidity when dm/dt was less than 0.001% in 30 s. The sorption and desorption sequence was set to repeat four times on each sample (without changing the powder sample), and was repeated on at least two different samples for each mixture content.

3. Results

Despite its aqueous solubility, α-lactose monohydrate is not a hygroscopic material. Indeed, the adsorption of water at humidities up to 80% RH amounts to as little as 0.02% weight increase (see results and discussion below). It follows that lac-

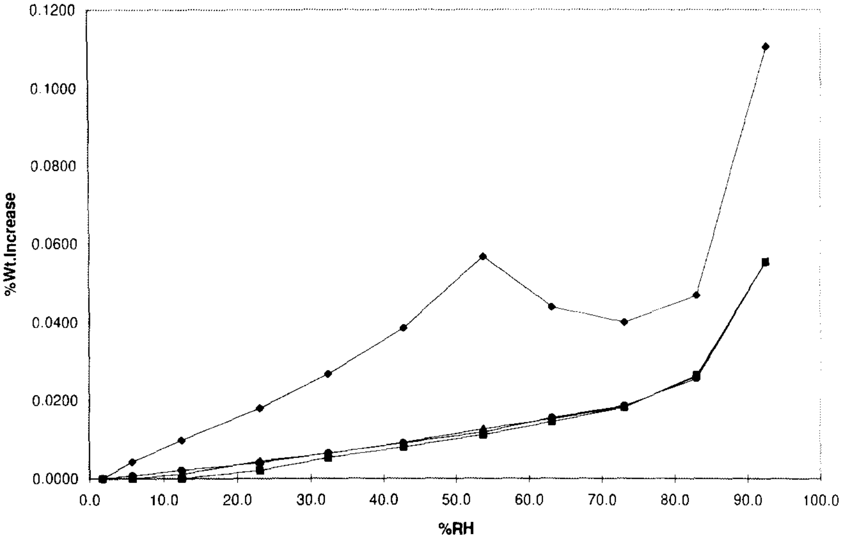

Fig. 1. Sorption isotherms for four repeat cycles on the same sample of a mixture, which initially contained 0.500% spray dried (amorphous) lactose, with the remainder being pre-conditioned crystalline α-lactose monohydrate (◆ = first cycle, then ■, ▲, ●).

tose is a difficult sample to investigate by gravimetric water sorption experiments and as such provides a harsh test for the equipment that has been utilised in this study.

Data for the sorption phases of multi-cycle experiments are presented in Fig. 1–4 for mixtures containing 0.5, 0.25, 0.125 and 0.05% amorphous material, respectively. In each case it can be seen that there is a difference between the first cycle and the other cycles. All cycles after the first show superimposable responses. The higher weight increases for the first sorption cycles of each mixture are a consequence of water being absorbed into the amorphous regions of the

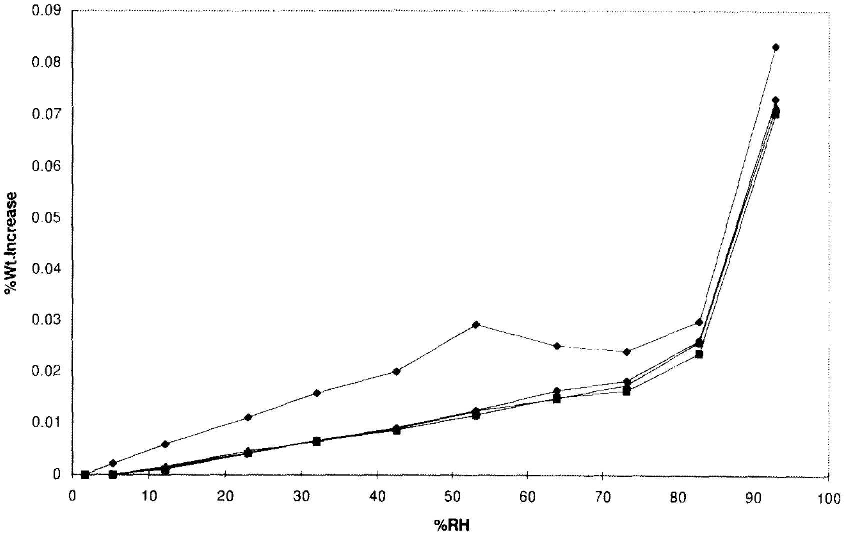

Fig. 2. Sorption isotherms for four repeat cycles on the same sample of a mixture, which initially contained 0.250% spray dried (amorphous) lactose, with the remainder being pre-conditioned crystalline α-lactose monohydrate (key as Fig. 1).

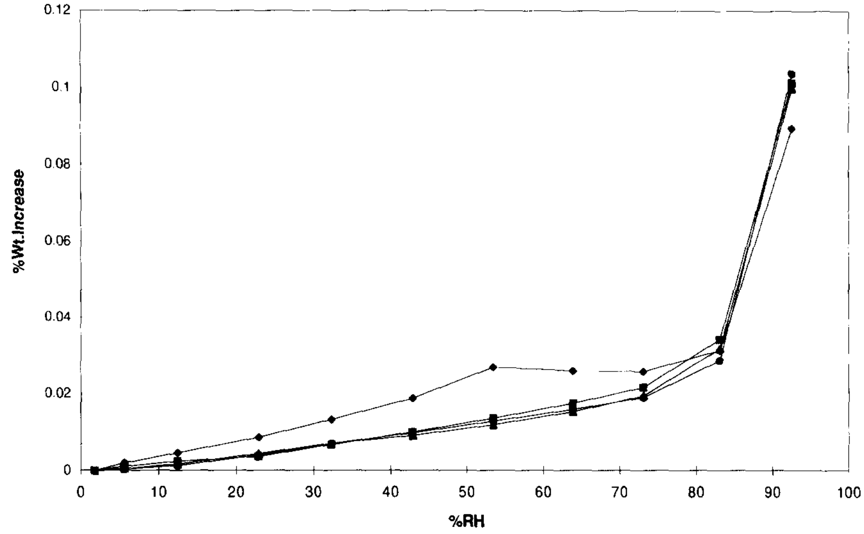

Fig. 3. Sorption isotherms for four repeat cycles on the same sample of a mixture, which initially contained 0.125% spray dried (amorphous) lactose, with the remainder being pre-conditioned crystalline α-lactose monohydrate (key as Fig. 1).

samples as well as being adsorbed onto the surface of the crystalline material. In each case (Fig. 1–4) the increase due to absorbed water is observed from the lowest humidities, but in many cases the isotherm joins the responses observed for the subsequent cycles at high humidities. The reason for this is that the first cycle shows the process whereby the absorbed water causes the amorphous material to recrystallise, thus there is an increase in weight due to absorption, causing the amorphous regions to be plasticised such that the glass transition temperature falls to that of the experiment (25° C), allowing sufficient freedom of molecular movement to facilitate crystalli-

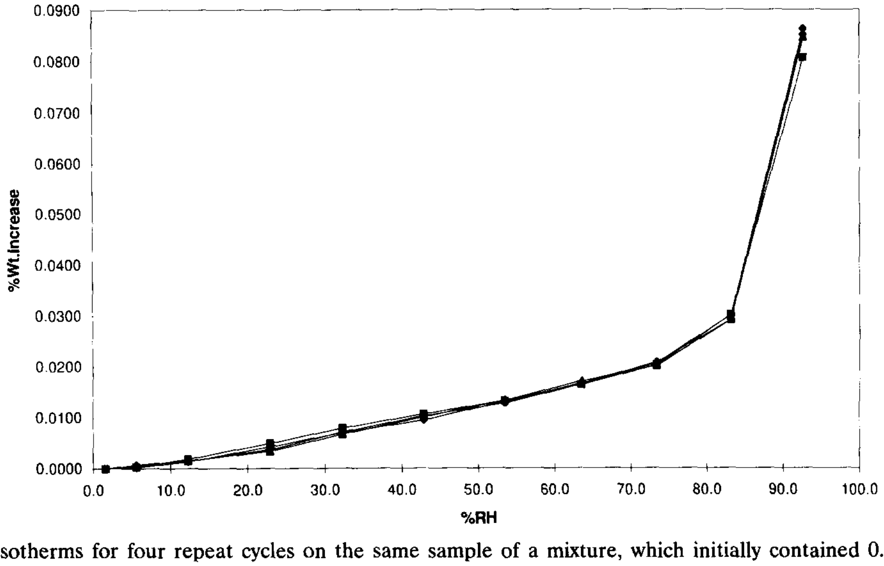

Fig. 4. Sorption isotherms for four repeat cycles on the same sample of a mixture, which initially contained 0.050% spray dried (amorphous) lactose, with the remainder being pre-conditioned crystalline α-lactose monohydrate (key as Fig. 1).

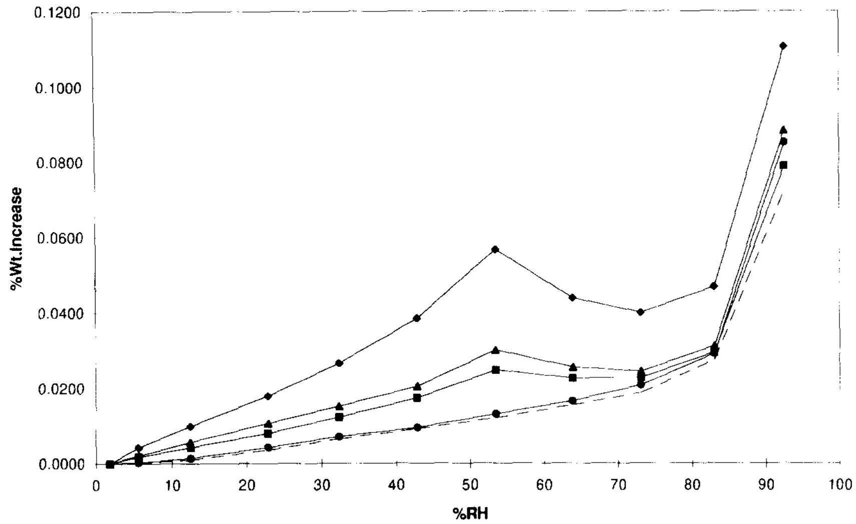

Fig. 5. Mean sorption isotherms for all repeat samples containing 0.050 (●), 0.125 (■), 0.250 (▲) and 0.5 (◆) % amorphous lactose, along with the mean of all adsorption responses (the second, third and fourth cycles for each of the samples) (dashed line without symbols).

sation. Upon crystallisation the water that was absorbed is expelled from the material with subsequent weight loss.

There are clear differences between the responses in Fig. 1–4, in that the greater the amorphous content the greater the area enclosed between the first sorption response and the subsequent adsorption events. These differences are highlighted in Fig. 5, which is a composite of the mean values for the first sorption isotherms for the repeat samples of each different mixture, and all the subsequent adsorption experiments grouped together as one data set (i.e., the mean of the second, third and fourth adsorption isotherms for every data set, irrespective of the amorphous content of the initial mixture). However, as the first response is a composite of absorption followed by water expulsion after crystallisation, the area between the first and subsequent isotherms reflects a kinetic contribution for the recrystallisation as well as the amount of amorphous material that was present. As such it is not simple to estimate the amorphous content for an unknown sample by taking a weight differential at, for example, a certain humidity. However, the increase in weight from the starting point of the first sorption run to the end of the first desorption run may be a useful approach to

Table 1

The theoretical increase in weight of samples containing various amounts of amorphous material, after sorption, crystallisation (to convert the amorphous material into the monohydrate) and desorption (to remove non-stoichiometric water), compared with that which was measured experimentally

Amorphous content (%)	Theoretical increase (%)	Measured increase (% ± SD)	Calculated amorphous content (%)
0.500	0.026	$0.025 \pm 1.6 \times 10^{-3}$ ($n = 2$)	0.475
0.250	0.013	$0.012 \pm 2.5 \times 10^{-3}$ ($n = 7$)	0.228
0.125	0.007	$0.010 \pm 4.3 \times 10^{-3}$ ($n = 7$)	0.190
0.050	0.003	$0.005 \pm 4.7 \times 10^{-3}$ ($n = 3$)	0.095

The final column is the calculated amorphous content for the samples, assuming the mean data to be a true reflection of the amorphous content and that all the amorphous material converts to the monohydrate.

the characterisation of the amorphous content. This is because the amorphous lactose crystallises to form a monohydrate under the conditions of this experiment (due to plentiful water vapour, possibly aided by the presence of α-lactose monohydrate seeds). The molecular weight of lactose monohydrate is 360, of which 5% is the water of crystallisation. Consequently, if a totally amorphous sample were investigated, and if it recrystallised to from the monohydrate then the weight gain after desorption (of physically adsorbed water, but not stoichiometric hydrate water) would be 5.26%. Similarly, the theoretical weight increases, expected due to the addition of water of crystallisation to the samples used in this study, have been calculated and are presented in Table 1. For a sample weight of 50 mg (as used in this study, although larger samples could be investigated) and with a detection sensitivity on the balance of 0.1 μg it is well within the instrument's capability to detect residual weights of the magnitude of those in Table 1.

The data for the residual weights obtained from the difference between the starting weight and the weight at equilibrium after the first desorption cycle has been brought down to 5% RH (see, for example, Fig. 6) are presented in Table 1. The choice of 5% RH as a point to terminate the desorption isotherm was to get a reproducible value, as it was found that storage at 0% RH gave considerable variability, presumably due to loss of some water of crystallisation. Surprisingly, in contrast to the sample on the desorption isotherm, the original material was stable to 0% RH air for at least 1 h, during which there was no evidence of loss of hydrate water. It can be seen (Table 1) that reasonably good agreement exists between the practical and theoretical data. The standard deviations of the data sets, and the difference between the theoretical and practical values are inversely proportional to the amorphous content of the mixtures. Consequently, it is reasonable to assume that the deviation from the theoretical data for the mixtures with a very small content of amorphous material is to a large extent due to the errors associated with weighing, mixing and sampling the extremely small quantities of amorphous lactose. Having noted these points, the residual water content remains a less than totally reliable method for accurate quantification of the degree of crystallinity for this material. For materials which do not crystallise to a stoichiometric hydrate, even though it would be simple to identify the existence of the amorphous material, alternative approaches would have to be investigated for quantification. Materials which are not

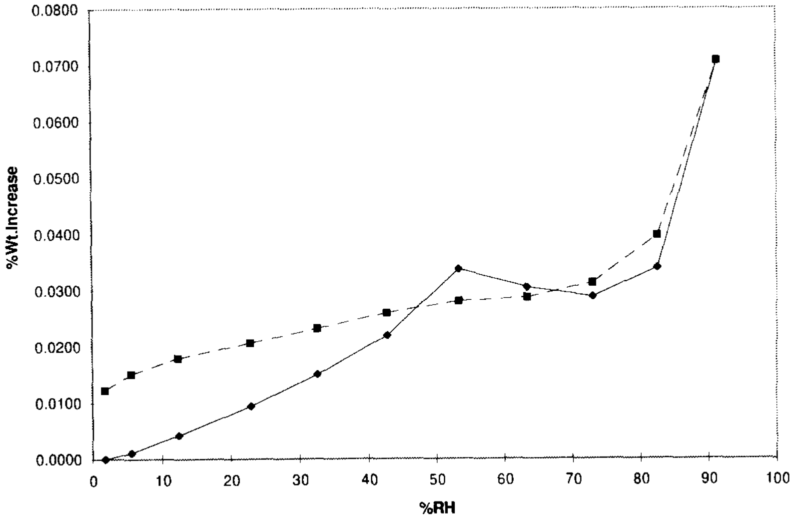

Fig. 6. A typical response for the first sorption cycle and subsequent desorption. This example is for 0.25% amorphous lactose and the retained weight on desorption to 5% RH is 0.012%.

freely soluble in water will be best investigated using a non-aqueous vapour in the experiment in order to induce crystallisation.

Despite the problems with quantification, the ability to distinguish between samples with such small amounts of amorphous content of lactose is a major advance in the field and demonstrates the value of accurate water sorption equipment for studies such as this.

References

Briggner, L.-E., Buckton, G., Bystrom, K. and Darcy, P., The use of isothermal microcalorimetry in the study of changes in crystallinity induced during the processing of powders. *Int. J. Pharm.*, 105 (1994) 125–135.

Buckton, G., Choularton, A., Beezer, A.E. and Chatham, S.M., The effect of comminution technique on the surface energy of a powder. *Int. J. Pharm.*, 47 (1988) 121–128.

Buckton, G., Darcy, P. and MacKellar, A., The use of isothermal microcalorimetry in the study of the study of small degrees of amorphous content in powders. *Int. J. Pharm.*, 117 (1995b) 253–256.

Buckton, G., Darcy, P., Greenleaf, D. and Holbrook, P., The use of isothermal microcalorimetry in the study of changes in crystallinity of spray dried salbutamol sulphate. *Int. J. Pharm.*, 116 (1995a) 113–118.

Elamin, A.A., Alderborn, G., Nystrom, C. and Ahlneck, C., Effects of mechanical processing on the solubility of griseofulvin. *AAPS Annual Meeting, San Antonio*, 1992, p. 163 (PT6196).

Florence, A.T. and Salole, E.G., Changes in crystallinity and solubility on comminution of digoxin and observations on spironalactone. *J. Pharm. Pharmacol.*, 28 (1976) 637–642.

Grant, D.J.W. and York, P., A disruption index for quantifying the solid state disorder induced by additives or impurities: II. Evaluation from heat of solution. *Int. J. Pharm.*, 28 (1986) 103–112.

Hendriksen, B.A., Characterisation of calcium fenoprofen 1. powder dissolution and degree of crystallinity. *Int. J. Pharm.*, 60 (1990) 243–252.

Otsuka, M. and Kaneniwa, N., Effect of grinding on the crystallinity and chemical stability in the solid state of cephalothin sodium. *Int. J. Pharm.*, 62 (1990) 65–74.

Pikal, M.J., Lukes, A.L., Lang, J.E. and Gaines, K., Quantitative crystallinity determination of β-lactam antibiotics by solution calorimetry: correlation with stability. *J. Pharm. Sci.*, 67 (1978) 767–772.

Saleki-Gerhardt, A., Ahlneck, C. and Zografi, G., Assessment of disorder in crystalline solids. *Int. J. Pharm.*, 101 (1994) 237–247.

Sebhatu, T., Angberg, M and Ahlneck, C., Assessment of the degree of disorder in crystalline solids by isothermal microcalorimetry. *Int. J. Pharm.*, 104 (1994) 135–144.

Sheridan, P.L., Buckton, G. and Storey, D.E., Comparison of two techniques for the assessment of the wettability of pharmaceutical powders. *J. Pharm. Pharmacol.*, 45 (1993) 1P.

Thompson, K.C., Draper, J.P., Kaufman, M.J. and Brenner, G.S., Characterisation of the crystallinity of drugs: B02669, a case study. *Pharm. Res.*, 11 (1994) 1362–1365.

Ticehurst, M.D., Rowe, R.C. and York, P., Determination of the surface properties of two batches of salbutamol sulphate by inverse gas chromatography. *Int. J. Pharm.*, 111 (1994) 241–249.