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Determination of low levels of amorphous content in inhalation grade lactose by moisture sorption isotherms

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ABSTRACT

α-Lactose monohydrate is widely used as an excipient in dry powder inhalers, and plays a very important role in the efficiency of the drug delivery. Due to the processing, low levels of amorphous lactose could be present in the blends. Varying amounts could have a strong effect on the efficiency of drug delivery of the powder blends. Therefore, the accurate measurement of low levels of amorphous lactose content is very important. A new method was developed to measure the amorphous content, based on dynamic vapour sorption (DVS). In contrast to the traditional re-crystallization approach of amorphous lactose, the new method is based on moisture sorption isotherms. Moisture sorption isotherms of blends of crystalline α-lactose and freeze-dried or spray-dried amorphous lactose were measured. By fitting the data with a Brunauer, Emmett, and Teller (BET) isotherm, a linear correlation was found between measured and actual amorphous content for the whole range of 0.1–100%. Differences between freeze-dried and spray-dried lactose, due to different molecular arrangements, could be removed by a preconditioning the samples at 35% RH prior to the isotherm measurement. It was shown that accurate determination of very low concentrations of amorphous lactose content is possible using moisture sorption isotherm analyses.

1. Introduction

In respiratory delivery of drugs, crystalline lactose is one of the most commonly used excipients (Son and McConville, 2008). Its function is twofold: as a bulking agent and to facilitate the aerosolisation of the drugs (Steckel et al., 2004). Impurities present in lactose can have a strong effect on the efficiency of the delivery of the drugs to the lungs. A special kind of impurity is amorphous lactose (Hancock and Zografi, 1997), presence of which in crystalline lactose could be caused by several reasons. For instance, during the drying process after the crystallization amorphous regions on the crystals may be formed from residual lactose dissolved in the adhering mother liquor. Also by processing steps such as blower transport or by milling of the crystals, amorphous lactose can be formed (Willart et al., 2004). Although the function of amorphous lactose related to the efficiency of delivery of drugs to the lungs is still unclear, it is obvious that the levels of amorphous content should be controlled (Zeng et al., 2007). Besides that, amorphous lactose has been linked to instability and moisture uptake of lactose powder blends (Das et al., 2009). Therefore, the presence of amorphous lactose in inhalation grade powder blends should be limited to minimal levels. From an understanding point of view, it is of great importance to be able to analyze the amorphous content at very low levels with a reliable and accurate method. Also from regulatory and quality assurance perspectives, it is of great importance to measure the amorphous content accurately. Furthermore, the amorphous lactose is in a metastable state and as such it will be prone to changes in time, possibly initiated by changes in relative humidity and/or temperature.

A number of techniques have been developed that are used to analyze the amorphous content (Buckton and Darcy, 1999; Lehto et al., 2006; Shah et al., 2006). A gravimetric approach such as dynamic vapour sorption (DVS) developed by Buckton and Darcy (1995) and Burnett et al. (2009), utilizes the mass increase as a result of the crystallization of amorphous lactose to crystalline α-lactose monohydrate. The crystallization is induced by exposure of a sample to water vapour. Water adsorption in amorphous lactose has a strong matrix plasticizing effect, resulting in a strong decrease of the glass transition temperature with increasing water activity. High relative humidity conditions (i.e. RH > 58% at room temperature (Timmermann et al., 2006)) give rise to a strong increase in molecular mobility in amorphous lactose allowing spontaneous crystallization to thermodynamically more stable crystalline forms of lactose (Buckton and Darcy, 1996). After crystallization, the excess water present in the now crystalline material is removed by drying and from the mass difference before and after the

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Nomenclature

BET	sorption isotherm equation according Brunauer, Emmet, and Teller
GAB	sorption isotherm equation according Guggenheim, Andersen, and de Boer
RH	Relative Humidity
P	partial water vapour pressure
P_0	vapour pressure of pure water
a_w	water activity
W	moisture content [kg kg^{-1}]
W_m	monolayer water content [kg kg^{-1} dry basis]
W_m(AC)	monolayer water content at a certain amorphous content (AC)
W_m(100)	monolayer water content at 100% amorphous content
$\overline{W_m(100)}$	concentration weighted average value of the monolayer water content
C	BET/GAB constant [dimensionless]
k	GAB constant
$M_w(H_2O)$	molecular weight of water
M_w(lactose)	molecular weight of lactose
FD	freeze-dried
SD	spray-dried
AC	amorphous content [%w/w]
DVS	dynamic vapour sorption
R^2	the square of the correlation coefficient

crystallization the amorphous content can be calculated, assuming that solely α-lactose monohydrate is formed. This is a sensitive method (Burnett et al., 2009) and the level of quantification is about 0.5%. Furthermore, this method is very reproducible and it is used in general to access the amorphous content. However, the mentioned assumption in this method that all amorphous lactose crystallizes completely to α-lactose monohydrate may not always be valid. Several reports show the formation of other crystalline forms of lactose (Kirk et al., 2007) such as anhydrous β-lactose and anhydrous α-lactose during the crystallization event (Buckton and Darcy, 1999; Timmermann et al., 2006). This phenomenon will reduce the measured amount of amorphous lactose with an unknown quantity. From regulatory, understanding, and quality points of view this is an unwanted effect.

Spectroscopic techniques such as Raman (Katainen et al., 2005), solid state NMR (Shah et al., 2006; Geppi et al., 2008), X-ray diffraction (Chen et al., 2001), and near infrared spectroscopy (Hogan and Buckton, 2001) have been used to study the amorphous content of pharmaceutical powders. Although these are in general non-destructive techniques, their sensitivity is not high enough to be able to measure the very low levels of amorphous lactose present in crystalline grades that are used for inhalable applications (Lehto et al., 2006). X-ray diffraction is a sensitive technique and levels of quantification of 1% have been reported (Chen et al., 2001). For an advanced Raman spectroscopy technique, a level of detection of 1.7% and a level of quantification of 5% were described (Whiteside et al., 2008). Calorimetric techniques have been described (Listiohadi et al., 2009) and these also lack a high sensitivity. Furthermore, these need a careful calibration in order to be able to quantify the amorphous content (Dilworth et al., 2004).

Here we will describe a new method that is based on the gravimetric moisture sorption technique. Instead of assessing the phase transition of amorphous to crystalline α-lactose monohydrate, it utilizes the water sorption behaviour of amorphous lactose. Water sorption by amorphous lactose is a well-described phenomenon in the dairy industry, because lactose is a major component of milk and related powders (Omar and Roos, 2007; Listiohadi et al., 2005). Several moisture sorption isotherms exist that are used to describe the relation between water content and water activity (a_w) (Al-Muhtaseb et al., 2002; Jouppila and Roos, 1997). Water activity can be defined as:

$$a_w = \frac{p}{p_0} = \frac{\mathrm{RH}}{100\%} \quad (1)$$

Where p is the partial water vapour pressure, p_0 the vapour pressure of pure water, and RH is the relative humidity. The Brunauer, Emmett, and Teller (BET) and the Guggenheim, Anderson, and de Boer (GAB) isotherms are the most widely used equations (Timmermann et al., 2001). The GAB equation can be expressed as follows:

$$\frac{W}{W_m} = \frac{Cka_w}{(1-ka_w)(1-[1-C]ka_w)} \quad (2)$$

with W the moisture content, W_m the monolayer moisture content, C and k are constants that are related to interaction energy of the different sorption layers. Mathematically BET and GAB are closely related. In the special case of $k = 1$ the GAB equation reduces to the BET equation. Originally, the BET isotherm was developed to describe the adsorption behaviour of vapours to surfaces. It is a multilayer adsorption model and it allows to make an estimate of the number of adsorption sites in a monolayer and from that the specific surface area of the sample. The BET expression treats the first and following layers equally whereas the GAB equation takes better into account the differences in interaction between the first and further layers. Therefore, the BET is only valid at the lower relative pressure region ($0.05 < a_w < 0.4$) where mainly the first layer is covered.

The sorption isotherms of amorphous lactose are well described (Bronlund and Paterson, 2004; Omar and Roos, 2007). At low a_w values, the sorption follows a BET like isotherm. In this region the material starts to sorb water, first in a monolayer around the lactose molecules. At higher a_w values, water is sorbed in multilayers and due to the increasing amount of water, the molecular mobility starts to increase, known as the plasticizing effect of water in glassy materials (Karel and Lund, 2005). When the glass transition temperature of the amorphous lactose is decreased close to or below the temperature of the environment, spontaneous crystallization occurs. This event was observed by atomic force microscopy (Price and Young, 2004). They observed incomplete crystallization at RH 58% and 75% and that a RH of 94% was required to obtain more or less complete crystallization. After crystallization, the sorption capacity of the crystalline lactose is much lower and it will expel water, resulting in a decrease in water content and therefore a decrease of mass. The crystallization event is irreversible under these conditions and an additional cycle of drying and moistening will result in a sorption isotherm of crystalline lactose which sorbs hardly any water. BET isotherms of amorphous lactose have been reported and a monolayer moisture content of 4.9–6.9 has been reported by various authors (Bronlund and Paterson, 2004; Jouppila and Roos, 1994).

Bronlund and Paterson (2004) described the moisture sorption isotherms for amorphous lactose. They used a range of saturated salt solutions in order to obtain a relative humidity varying from 0 to 60%. Above ~60% RH at room temperature the material crystallizes. The obtained isotherms were described with a GAB equation. Although low levels of amorphous content were detected, only a relationship between isotherm parameters and amorphous content was predicted and not reported. Organic vapour sorption was carried out on lactose samples (Young et al., 2007) and a linear relationship between mass increase at a certain relative vapour pressure and amorphous content was reported. The disadvantage

of this method is the mismatch between lactose and the organic vapours. Lactose particles will only sorb the octane vapours on or close to the surface and differences in particle size could give strong differences in organic vapour sorption.

The method we present is capable of quantifying the amorphous lactose content accurately over the whole range of 0–100% amorphous lactose, including the low level range of 0.1–1%. This is done by measuring the moisture sorption isotherms of lactose with known amorphous content and correlating the obtained BET parameters (monolayer water content W_m) with the amorphous content. It was chosen to take two ways to prepare amorphous lactose because it is known that differently prepared amorphous lactose can behave differently in moisture sorption experiments (Craig et al., 2001). For calibration of the measurements, blends were prepared of crystalline lactose with spray-dried or with freeze-dried lactose as the amorphous phase.

2. Materials and methods

2.1. Materials

2.1.1. Crystalline lactose

Lactohale® and Lactochem® pharmaceutical grade α-lactose monohydrate (FrieslandCampina DOMO, The Netherlands) were used as crystalline lactose. To ensure a completely crystalline lactose, it was stored for 48 h at 25 °C and 80% RH in a temperature and relative humidity controlled chamber (SPS-11 10 μ Automated Sorption Test System, Projekt Messtechnik, Ulm, Germany). Subsequently, this material was conditioned to 30% RH at 25 °C in the same climate controlled chamber and stored in sealed aluminium containers at room temperature prior to further analyses.

2.1.1.1. Freeze-dried amorphous lactose, method A. Lactochem® pharmaceutical grade α-lactose monohydrate (FrieslandCampina DOMO, The Netherlands) was dissolved in demineralised water during 1 h at room temperature to obtain a clear 15% (w/w) lactose solution. The lactose solution was divided in 50 mL portions in plastic bags suitable for freezing. Each bag was immersed in liquid nitrogen during 2 min to ensure fast pre-freezing of the lactose solution. The frozen solution was collected from the plastic bags and transferred into glass vials and connected to a Virtis Freezemobile 25XL freeze dryer (The Virtis Company, New York, USA). The freeze dryer was set at −72 °C, and the amorphous lactose was dried during 4 days at 8 mTorr. The freeze-dried lactose was subsequently stored in sealed containers at room temperature prior to further analyses. The complete amorphicity of the lactose was verified by X-ray diffraction and optical microscopy as described in Section 2.2.1.

2.1.1.2. Freeze drying method B. A solution of 15% (w/w) Lactohale® 100 (DOMO, The Netherlands) in double distilled water was prepared and 1.5 mL of the solution was filled into 2R glass vials each. Pre-freezing of the solution was carried out in a freeze-drier Alpha 1-4 (Christ Gefriertrocknungsanlagen GmbH, Germany) at a temperature of −25 °C. For main drying of the samples, the temperature was set to −27 °C corresponding to a pressure of 0.52 mbar in the freeze drying chamber. Afterwards, the temperature was set to −13 °C and stepwise increased to 30 °C while the pressure reached values of 1.98 mbar for a final drying step. The complete amorphicity of the freeze-dried lactose samples was detected with X-ray diffraction measurements as described in Section 2.2.1. Samples with a definite amount of freeze-dried lactose for the determination of amorphous content were prepared by sandwich weighing the crystalline and freeze-dried lactose directly into the Cahn-balance of the DVS

2.1.2. Spray-dried (amorphous) lactose

Amorphous lactose was produced by spray drying a 15% (w/w) Lactohale® 100 solution in double distilled water with a Büchi mini spray dryer B-290 (Büchi Labortechnik GmbH, Germany). The process was carried out at standard conditions: inlet temperature was adjusted to 152 °C and outlet temperature to 70 °C by varying the pump rate. After spray drying, the complete amorphicity of the lactose was verified by X-ray diffraction as described in Section 2.2.1.

2.1.3. Powder blends of amorphous and crystalline lactose

2.1.3.1. Blends with freeze-dried lactose. Powder blends of amorphous freeze-dried lactose and crystalline lactose were prepared by adding appropriate amounts of both forms in the sample cup of the SPS-11 10 μ Automated Sorption Test System (Projekt Messtechnik, Ulm, Germany). By blending, samples were obtained with known amounts of amorphous content varying from 0 to 100% (w/w). Since the possible sample size for analysis in this equipment is relatively large (typically 800–1200 mg) it is well possible to prepare samples accurately using a microbalance (Mettler-Toledo AE163, 10 μg precision). The freeze-dried amorphous lactose flakes were ground by hand in a mortar directly before weighing. The amorphous lactose and crystalline lactose were mixed in the sample cup using a spatula and it was made sure that no powder remained on the spatula after mixing. All sample preparations were carried out in an air conditioned laboratory at 20 °C and a RH well below 45%. Exposure of the powders to the environment was kept as short as possible to exclude crystallization of the amorphous lactose.

2.1.3.2. Blends with spray-dried lactose. Powder blends of amorphous spray-dried lactose and crystalline lactose were prepared by weight addition and subsequent dry blending using a Turbula blender (Type T2C, Bachofen AG, Switzerland). The (amorphous) spray-dried and crystalline powders were sieved separately (425 μm sieve) and weighed into a stainless steal mixing vessel using a "sandwich" weighing procedure to obtain samples with known amount of amorphous content varying from 0 to 100% (w/w). After weighing, the components were blended for 15 min in the Turbula blender, subsequently the mixture was sieved (425 μm) and blended again for 15 min. A final sieving step (425 μm) was added prior to filling the mixture into airtight aluminium containers. The weighing and sieving steps as well as the filling into aluminium containers were carried out under nitrogen atmosphere in a glove box below 25 °C and 25% RH to avoid crystallization of amorphous lactose.

2.2. Methods

2.2.1. X-ray diffraction and optical microscopy

X-ray diffraction to verify complete amorphicity of the spray-dried and freeze-dried lactose is carried out using a Stoe X-ray diffractometer (Stoe & Cie, Germany) by analyzing a 2θ range from 5° to 50°. If only the characteristic Halo signal is detected in the diffractograms, it can be stated that the sample is fully amorphous. Additionally, the amorphous character of the powder was confirmed using an Olympus BX51 bright field light microscope equipped with a polarisation filter. The lactose was dispersed in immersion oil with a refractive index of 1.516 (Merck, Darmstadt, Germany) and observed at 10× and 40× magnification using polarised light. No bright domains were observed indicating that no crystalline material was present in the amorphous lactose samples.

2.2.2. Specific surface area

The specific surface area of crystalline, spray-dried and freeze-dried lactose powders was determined with a BET gas adsorption method. Degassing of the lactose powders was completed for 1 h

under vacuum at 40 °C prior to analyzing the samples with a Gemini 2360 BET surface area analyzer (Micromeritics, Norcross, USA) using nitrogen as measuring gas. The BET multipoint method was used to calculate the specific surface area. All measurements were carried out in triplicate.

2.2.3. Dynamic vapour sorption—crystallization approach

Dynamic vapour sorption experiments were carried out in a DVS 1 (Surface Measurement Systems Ltd., UK) using a gravimetric approach to determine the amorphous content, similar to the method described by Buckton and Darcy (1995). Approximately 50 mg of the powder mixtures was weighed into a Cahn-balance and dried in a dry nitrogen flow at almost 0.0% RH until an equilibrium of mass was reached (after 240 or 360 min). This equilibrium mass was used as dry mass for the calculation of amorphous content. The drying step was followed by a wetting step of the lactose sample for 240 min in a nitrogen flow saturated with water, so that a RH of 90.0% was achieved. During this wetting process the amorphous lactose present in the sample crystallized. Then, again, the sample was dried for 240 min or 360 min at almost 0.0% RH until an equilibrium of mass after crystallization was reached. The second equilibrium mass is labelled as end mass to calculate the amorphous content of the sample. By calculating the difference between dry mass and end mass, the amount of α-lactose monohydrate created due to crystallization of amorphous parts could be estimated assuming that amorphous lactose is transferred into the monohydrate form only. Because of this stoichiometric formation of α-lactose monohydrate from amorphous lactose, the amorphous content (AC) originally present in the sample was calculated as follows:

$$\mathrm{AC}[\%w/w] = [M_w(\mathrm{lactose})] \cdot \frac{(\mathrm{End\_Weight} - \mathrm{Dry\_Weight})}{(\mathrm{Dry\_Weight} \cdot [M_w(\mathrm{H_2O})])} \times 100\% \quad (3)$$

where $M_w(H_2O)$ is the molar mass of water (18.02 g/mol), M_w(lactose) is the molar mass of lactose (342.3 g/mol), Dry_Weight is the sample weight at ~0% RH before crystallization, and End_Weight is the sample weight at ~0% RH after crystallization at 90% RH.

Determination of the amorphous content of the powder blends was carried out at least in triplicate, determination of the freeze-dried samples by this method was carried out once.

2.2.4. Moisture sorption isotherms

Moisture sorption isotherms of the powder blends were analyzed gravimetrically using an SPS-11 10 μ Automated Sorption Test System (Projekt Messtechnik, Ulm, Germany) equipped with a Mettler-Toledo SAG285 balance with 10 μg precision. In this equipment eleven samples can be monitored in parallel on a rotating disc. The aluminium sample cups have a diameter of ~5 cm, so that relatively large sample sizes can be used (800–1200 mg) while still having a monolayer of powder. As such, the SPS-11 is very suitable for heterogeneous samples as well. The relative humidity in the measurement chamber is conditioned by mixing of dried compressed air (DAS2 air dryer, Domnick Hunter Ltd., UK) at a flow rate of 1500 mL/min with vapourised demineralised water. Air distribution in the chamber is convectively controlled by multiple fans, while during weighing of a sample all air movement is stopped. The RH is monitored using a Rotronic sensor, and the temperature was always controlled at 25 °C.

Moisture sorption isotherms were analyzed after drying of the samples close to 0.0% RH for at least 20 h, followed by a stepwise increase of RH (3.0%; 5.0%; 7.5%; 10.0%; 15.0%; 20.0%; 30.0%; 40.0%). Each step was equilibrated for ~16 h, unless full mass equilibration was clearly reached much faster.

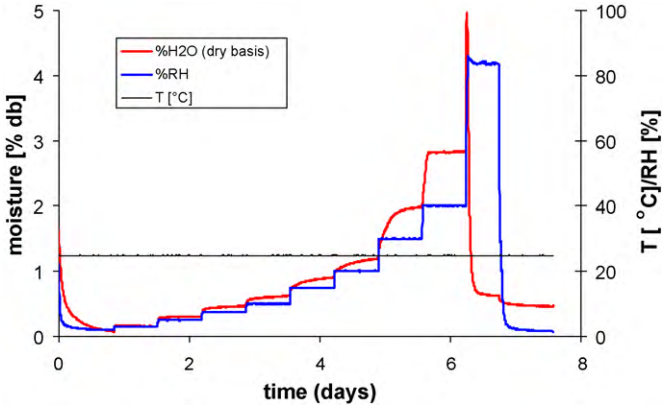

Fig. 1. Example of a moisture sorption isotherm measurement.

After the 40.0% RH step, the RH was increased to 85.0% RH to allow full crystallization of the amorphous lactose, followed by drying close to 0.0% RH in order to calculate the amorphous content according to the procedure described in Section 2.2.3. An example of a moisture sorption measurement is shown in Fig. 1.

2.2.5. Fitting procedure

Moisture sorption isotherms were described for each powder blend by fitting the equilibrium moisture sorption data in the a_w range between 0.0 and 0.4 [–] with the BET isotherm model (Eq. (2), with $k = 1$). For this, all moisture content data were always expressed on dry basis. The isotherm fit was optimised by the procedure of minimising the root of relative mean squared differences between data and model.

For optimisation, the monolayer moisture content (W_m), C, and the *dry mass* were used as fit parameters. The *dry mass* is the mass of the sample without free water. It was chosen as a fit parameter because complete drying could not be achieved and measurement of the free water content is not without error. However, the effect of this parameter was found to be small.

2.2.6. Determination of anomeric purity by gas chromatography

The anomeric purity of samples was determined according to the method of Dwivedi and Mitchell (1989). The lactose samples were derivatised using 1-(trimethylsilyl)-imidazole (Sigma–Aldrich, art. no. 394874) in anhydrous pyridine (Sigma–Aldrich, art. no. 27,0970). Analysis was carried out using a GC 6890N (Agilent, USA), equipped with a CIS-4 cooled injection system (Gerstel, Germany) and flame ionisation detection (Agilent, USA). A capillary column 19091S-433, HP-5ms, 0.25 mm × 30 m × 0.25 μm was used (Agilent, USA).

3. Results

3.1. Properties of amorphous and crystalline powders

The fully amorphous state of the samples was detected by powder X-ray diffraction. Both samples of freeze-dried lactose produced with different methods as well as the spray-dried lactose sample were found to be fully amorphous because only a characteristic Halo signal could be detected in the X-ray pattern (data not shown).

Prior to each moisture sorption experiment, the fully amorphous character of the SD and FD lactose was confirmed by optical microscopy using polarisation of the bright field light source. No bright domains were observed in the amorphous samples, whereas the birefringent crystalline lactose appeared as the typical bright tomahawk crystals. Determination of the specific surface area resulted in a higher value for the spray-dried amorphous lactose

than for either sample of freeze-dried lactoses. Values of about 1.2 m^2/g were obtained for the spray-dried lactose while values of 0.9 m^2/g (method A) and 0.2 m^2/g (method B) were found for the freeze-dried lactose. The preparation of freeze-dried lactose with method A led to a product with higher specific surface area than the preparation of freeze-dried lactose according to method B. It is expected that this will influence the moisture sorption kinetics but not the equilibrium moisture content, which is governed by the glassy matrix composition. The anomeric composition of both the SD and FD lactose was found to be 44% α-lactose and 56% β-lactose [w/w], which is close to the mutarotational equilibrium in solution (Roetman and Buma, 1974). The crystalline lactose had an α-lactose purity of >98% [w/w].

3.2. Moisture sorption of crystalline lactose

Moisture sorption isotherms were analyzed for the crystalline lactose, in order to be able to address baseline sorption behaviour. The moisture sorption isotherm of crystalline lactose was measured for different sample sizes (400 mg monolayer of powder and a 1500 mg heap of powder) and for two different sieve fractions (<80 μm and >80 μm). Since no significant difference in moisture sorption was detected between the various crystalline samples, all results were combined in a single generic moisture sorption isotherm which is shown in Fig. 2A. Fitting of this data resulted in a GAB isotherm (Eq. (2)) with $k = 0.87$, $C = 8.70$ and $W_m = 14.4 \times 10^{-3}$. In Fig. 2A it can be seen that the crystalline lactose does sorb some moisture, although absolute numbers are very small. This is in agreement with the results that were reported by Bronlund and Paterson (2004), who found that in the water activity range from 0 to 0.85 [–] crystalline lactose sorbed very little water. The same authors described an effect of packing density on the moisture sorption of crystalline lactose due to differences in capillary condensation effects, however this is only significant at $a_w > 0.85$ [–]. In the regime of interest for this research, i.e. $a_w \leq 0.4$, no difference between moisture sorption in different crystalline lactose samples was detected (Bronlund and Paterson, 2004). Although the baseline moisture sorption of the pure crystalline lactose is almost negligible, it is significant in low amorphous content blends. Therefore, the obtained data on the blends were corrected for the sorption of crystalline lactose, which mainly affected the low amorphous content powder blends.

3.3. Moisture sorption of freeze-dried lactose and of blends of freeze-dried and crystalline lactose

Moisture sorption isotherms of FD amorphous and crystalline lactose blends were determined. The whole amorphous content range of 0–100% was measured with emphasis on the low amorphous regime (0–10%). After baseline correction for moisture sorption by crystalline lactose, the isotherms were fitted using a BET moisture sorption model (Eq. (2), with $k = 1$). The BET fit parameters W_m and C are listed for all analyzed samples in Table 1 and the BET isotherms for some samples are plotted in Fig. 2B. With increasing amorphous content the moisture sorption capacity increased. In Fig. 2B it can be seen that even in the very low amorphous content regime significant differences in moisture sorption can be distinguished. Apparent is the fact that the monolayer moisture content value (W_m) proportionally increases with increasing amorphous content, and replicates show an excellent reproducibility of the W_m fit values. The BET fit factor C does not appear to be correlated with the amorphous content, and is in the same order of magnitude for the entire dataset. Independent replicates show significant fluctuations of fit factor C but not in W_m, which implies that C is of less importance for the absolute moisture sorption capacity of the freeze-dried lactose samples.

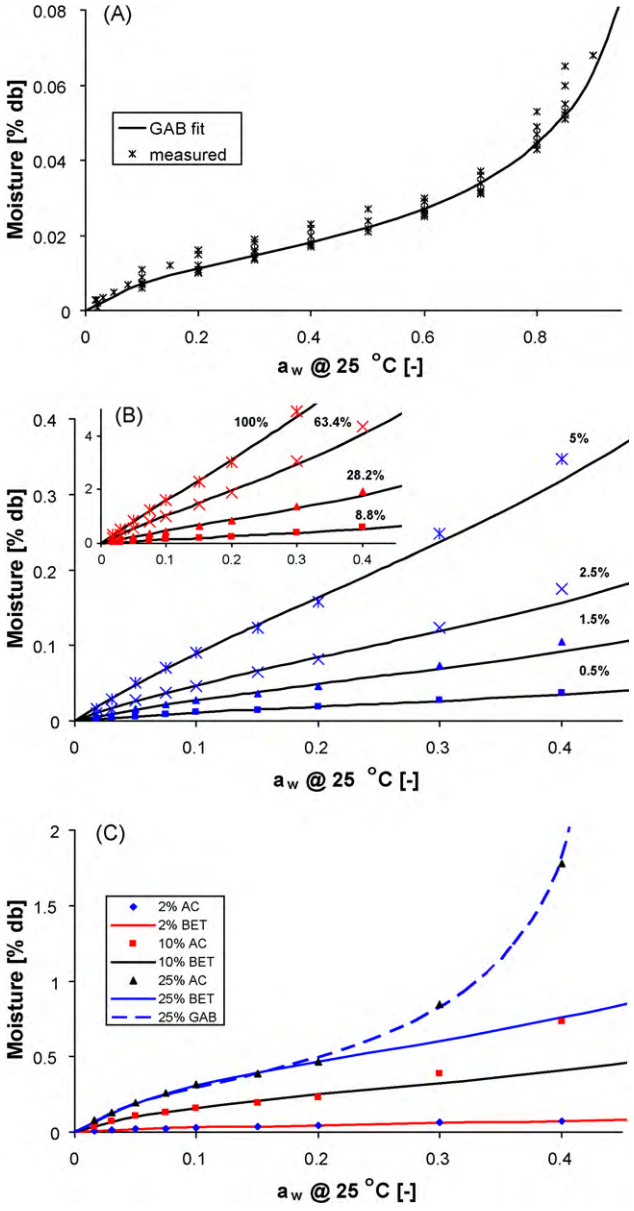

Fig. 2. Equilibrium moisture sorption isotherms and GAB or BET fits (straight lines) of (A) crystalline lactose fitted with a GAB model; (B) of FD amorphous and crystalline lactose blends with various amounts of FD amorphous (0.5–5%, inset 8.8–100% AC). BET model fits on data from $a_w = 0.05$–0.30 [–]; and (C) of SD amorphous and crystalline lactose blends with various amount of SD amorphous content. BET model fits on data from $a_w = 0.05$–0.20 [–].

Since the moisture sorption isotherms of the FD amorphous and crystalline lactose blends are corrected for the small effect of sorption by the crystalline fraction, the net effect of moisture sorption can be attributed to the amorphous lactose. As such, the W_m value of the amorphous fraction ($W_m(100)$) can be directly calculated from the added amorphous concentration (AC) and the analyzed W_m at that concentration ($W_m(AC)$):

$$W_m(100) = \frac{W_m(AC)}{AC} \times 100\% \quad (4)$$

The value of $W_m(100)$ was found to be consistent over the entire dataset, which means that the amorphous content in the blends of FD and crystalline lactose can be approached by a linear correlation with the W_m fit value ($R^2 = 0.999$), as is illustrated in Fig. 3A. This linear relation over the whole range of 0.1–100% also proofs that the blending procedure did not affect the actual amorphous content.

Table 1
BET fit parameters of blends of FD amorphous and crystalline lactose.

FD AC [%w/w]	W_m	C
0.13	0.008	9.4
0.26	0.017	3.6
0.35	0.020	4.8
0.50[b]	0.024 ± 0.003	6.7 ± 2.5
0.75	0.041	4.0
1.0[b]	0.054 ± 0.006	3.7 ± 0.7
1.5	0.073	4.6
2.5	0.12	4.7
5.0[b]	0.27 ± 0.006	3.2 ± 0.8
9.0	0.45	3.5
10.0[a]	0.55 ± 0.02	3.1 ± 0.4
16.5	0.90	3.3
22.9	1.29	3.2
28.2	1.57	3.2
43.1	2.40	3.2
56.9	3.10	3.3
63.4	3.61	3.1
71.0	4.17	3.0
90.7	5.36	3.0
100[c]	5.83 ± 0.2	3.0 ± 0.2

[a] Duplicates.
[b] Triplicates.
[c] Quadruples.

Since the low amorphous concentration regime is of most importance in the context of pharmaceutical excipients, a concentration weighted average value is calculated as:

$$\overline{W_m(100)} = \frac{\sum W_m(\mathrm{AC})}{\sum \mathrm{AC}} \times 100\% \quad (5)$$

For the FD and crystalline lactose blends the weighted average W_m is 5.74 with a standard deviation of 0.09. This is in good agreement with W_m values for freeze-dried lactose presented by several authors. Jouppila and Roos (1994) found 4.9–6.3 and Bronlund and Paterson (2004) reported values of 4.9–6.9 for freeze-dried lactose depending on the temperature. The amorphous content AC in a blend of FD and crystalline lactose can thus be calculated from the BET fit factor W_m(AC) of the analyzed moisture sorption isotherm as:

$$\mathrm{AC} = \frac{W_m(\mathrm{AC})}{5.74} \times 100\% \quad (6)$$

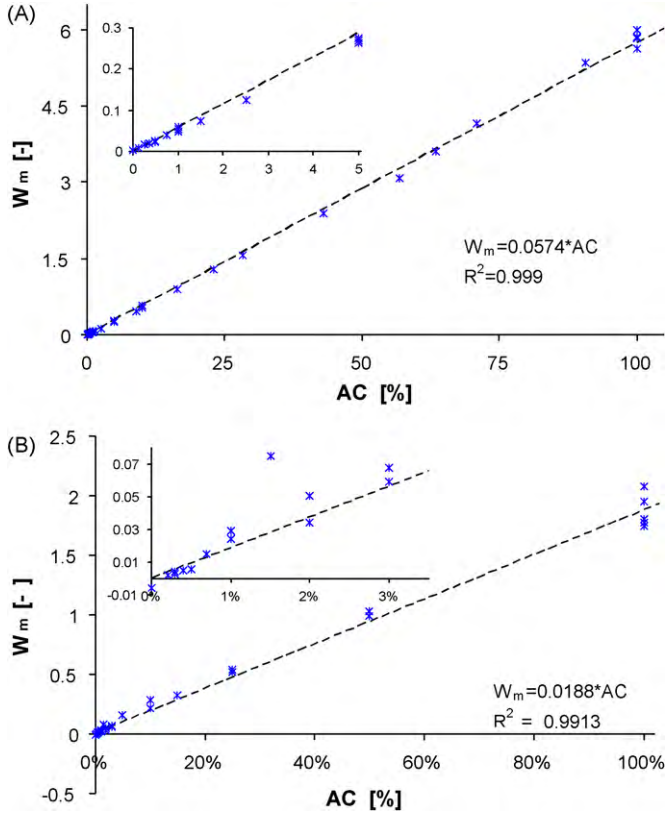

Fig. 3. Linear correlation between BET fit value W_m and FD amorphous content (A) or SD amorphous content (B) between 0 and 100% AC. The insets show the low AC regimes (0–5%).

3.4. Moisture sorption of spray-dried lactose and of blends of spray-dried and crystalline lactose

For the blends of SD amorphous lactose with crystalline lactose also the moisture sorption capacity increased with increasing amorphous concentration. In Fig. 2C the sorption data of some of the blends (2.0, 10.0 and 25.0% amorphous content) are plotted. Fitting all measured data points with a BET isotherm gave unsatisfactory results. The SD data show a more pronounced sigmoid sorption behaviour, with lower sorption capacity at $a_w \leq 0.2$ and a strong increase in equilibrium moisture content at $a_w > 0.2$. The latter suggests that a sorption model with stronger emphasis on multilayer sorption such as the GAB equation would be more suitable here. This is indeed the case as can be seen from the good GAB isotherm fit depicted by the dotted line in the 25% amorphous sample in Fig. 2C (Barbosa-Cánovas et al., 2007). Unlike the minor effect of the fit parameter C as described previously for the FD samples, variations in fit parameter k resulted in significant variations of W_m and thus the calculated amorphous content. In order to be able to obtain satisfactory moisture sorption isotherm fits by BET on the SD data, it was decided to only take data up to and including $a_w = 0.20$ into account. As such, a linear correlation between the added amorphous content (AC) and W_m fit was found ($R^2 = 0.991$), which is illustrated in Fig. 3B. The average value of the BET fit factor C was 65.1 and was not correlated to the amorphous content. Fluctuations in C were higher than observed in the FD lactose with a standard deviation of 12.0. The concentration weighted average W_m value ($\overline{W_m(100)}$, calculated according to Eq. (5)) was found to be 1.94 with a standard deviation of 0.12 (29 data points). Also here, the linear relationship indicates that the blending procedure did not alter the actual amorphous content. Although the linear correlation between W_m and the amorphous content in SD and crystalline lactose blends is rather good, the weighted average W_m is much lower than found for FD lactose and values reported in literature.

3.5. The effect of preconditioning at 35% RH on moisture sorption of spray-dried and freeze-dried lactose

Samples of 100% amorphous SD lactose and 100% amorphous FD lactose were subjected to a preconditioning step at 35% RH during 13 h prior to moisture sorption analysis as described in the previous sections. The obtained moisture sorption isotherms for both SD and FD lactose are shown in Fig. 5A. Both sorption isotherms are different from their original behaviour: the FD lactose showed a decrease and the SD lactose showed an increase in moisture sorption capacity. Interestingly, the moisture sorption isotherms of both the SD and the FD lactose are very similar after the preconditioning procedure, with GAB fit parameters for SD and FD being respectively $k = 1.41$ and 1.40, $C = 5.33$ and 5.86, and $W_m = 2.80$ and 2.73. This will be further elaborated in Section 4.

3.6. Amorphous content determination according the crystallization approach using DVS

The crystallization of spray-dried (SD) amorphous lactose in blends with crystalline lactose for the gravimetrical calculation of

Table 2
Added and measured amorphous content according to the re-crystallization approach (Buckton and Darcy, 1995).

Added (%)	AC[a] (%)	sd[b] (%)	AC/added[c]
0.10	0.10	0.08	1.0
0.20	0.05	0.05	0.25
0.30	0.13	0.17	0.42
0.40	0.22	0.15	0.53
0.50	0.24	0.06	0.48
1.0	0.43	0.09	0.43
2.0	0.68	0.24	0.34
5.0	1.69	0.13	0.33
10	3.85	0.60	0.38
15	5.20	0.07	0.34

[a] Measured AC.
[b] Standard deviation.
[c] Quotient of measured and added AC.

the amorphous content with dynamic vapour sorption as described in Section 2.2.3 led to well reproducible results (Table 2 and Fig. 4, series A). The linear fit of the calculated amorphous content with high coefficient of determination (R^2 = 0.9959) indicates a good homogeneity of the prepared powder blends. However, the calculated amorphous content of the powder blends containing SD amorphous lactose was found to be significantly lower than the actual amorphous addition of SD amorphous lactose to the powder blends prior to determination. Upon increasing added amorphous content, the underestimation of the measured amorphous content slightly increased, which clearly indicated the decrease in the quotient of added and measured amorphous content (Table 2). The use of freeze-dried lactose (prepared by method B) as amorphous component led to almost the same results using this method. Although a good linear correlation between the measured amorphous content could be seen, the measured amorphous content did not fit to the actual amorphous addition. Values in the same range of underestimation were calculated for the freeze-dried amorphous lactose in comparison to the values of spray-dried amorphous lactose. Similar results were described by Buckton and Darcy (1999). They found an underestimation of the measured amorphous content, which even increased at higher added amorphous contents. The reason for the observed underestimation is that not all of the amorphous lactose immediately re-crystallized to the α-monohydrate form (Buckton and Darcy, 1999). The formation of other polymorphic forms of lactose is very well possible (Timmermann et al., 2006). And this is probably enhanced by the presence of large amounts of β-lactose (54% by GC) in the amorphous phase that cannot or only

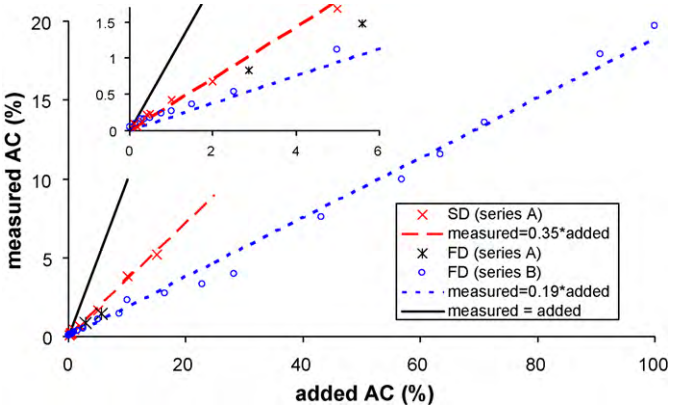

Fig. 4. Correlation between amorphous addition and analyzed amorphous content with DVS—crystallization approach. Series A as analyzed described in Section 2.2.3 on blends of crystalline lactose with SD and FD lactose, re-crystallization at 90%RH. Series B as analyzed described in Section 2.2.4 on blends of crystalline with FD lactose, re-crystallization at 85%RH. The inset shows the regime of 0–6% AC.

partially mutarotate to α-lactose before crystallizing. The blends of freeze-dried lactose (method A) with crystalline lactose that were analyzed on moisture sorption behaviour were also subjected to the re-crystallization approach as described in Section 2.2.4 and the results are illustrated in Fig. 4 (series B). A good linear correlation between added and analyzed amorphous content is found (R^2 = 0.9928). However, with on average only about 20% of added amorphous content measured, the underestimation is even more pronounced compared to the first described DVS method. This difference could be caused by the large differences in experimental setup and conditions in both methods (Timmermann et al., 2006).

4. Discussion

Moisture sorption isotherms were measured of mixtures of crystalline lactose with both freeze-dried and spray-dried amorphous lactose. Fitting of these isotherms with a BET formula gave a very good linear correlation between amorphous content and a fit parameter, i.e. the monolayer moisture content. Especially for amorphous lactose that was prepared by freeze drying, the linear correlation over the whole range of 0.1–100% amorphous content was excellent. In contrast to the re-crystallization approach that was developed by Buckton and Darcy (1995), all amorphous lactose could be assessed with this moisture sorption isotherm approach. However, large deviations in the sorption isotherms were observed as a function of the way of preparation of the amorphous lactose. The correlation factor for freeze-dried lactose was 5.74 whereas it was 1.94 for spray-dried amorphous lactose. The isotherms of the FD lactose blends gave good fits with a BET up to a_w = 0.4 whereas the SD lactose blends could only be fitted up to a_w = 0.2, after which large deviations from the BET fit were observed. It is not expected that the macroscopic structure of the powder particles influences the equilibrium moisture content of amorphous lactose at a given relative humidity. The most probable explanation for the observed differences between SD and FD lactose is the fundamentally different dehydration process leading to differences in the (metastable) thermodynamic properties of the solid glassy matrix. During spray drying most water removal occurs instantaneously after atomisation in the hot drying air, and macroscopically the drying particles can shrink almost proportionally with water removal. At molecular scale, the entire drying process occurs above glass transition temperature where molecular mobility is high. This is illustrated in Fig. 5B (Vuataz, 2002). As a result, lactose–lactose interactions can intensify especially in the final drying stage where the material is rubbery leading to a dense molecular arrangement in the spray-dried amorphous glassy matrix.

During freeze drying firstly the lactose solution is frozen, and during subsequent sublimation the solid structure does not shrink proportionally with water removal resulting in macroscopically porous particles. At molecular scale the mobility of the lactose molecules is very low during the entire drying process. The final drying stage takes place while the material is well below glass transition temperature, so that lactose–lactose interactions are limited. As a result, the free volume between amorphous lactose molecules in a freeze-dried glassy matrix is likely to be higher than in a spray-dried glassy matrix (Vrentas and Vrentas, 1991).

Moisture adsorption by these materials is expected to be different in the low relative humidity regime, since the freeze-dried matrix with a more open molecular arrangement with high free volume has more sites for water molecules to adsorb than the dense spray-dried lactose glass (illustrated in Fig. 5C). With increasing relative humidity the amorphous lactose approaches glass transition resulting in a sudden increase of molecular mobility (Burnett et al., 2004). Relaxation of the lactose molecules will take place, and it is expected that most of the differences in initial drying kinetics are therewith diminished. Interestingly, the moisture sorption

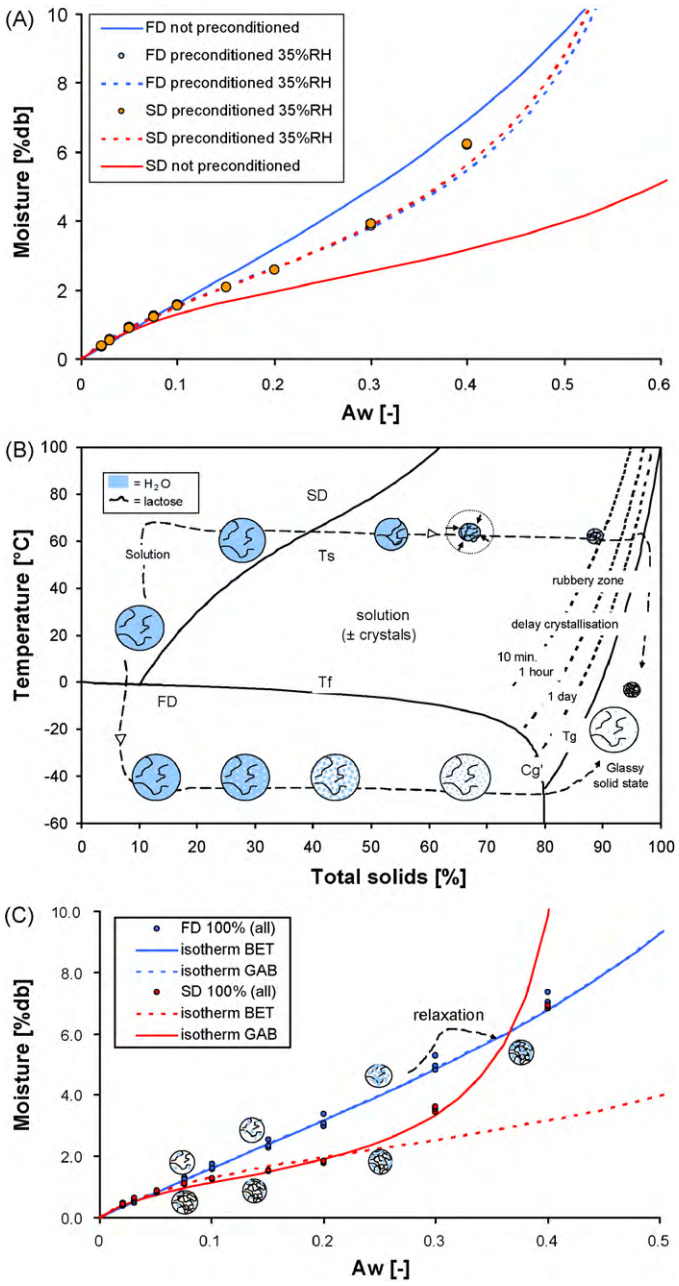

Fig. 5. (A) Moisture sorption isotherms of SD and FD lactose after preconditioning at 35% RH during 13 h. (B) Phase diagram of lactose and illustration of difference between SD and FD (Vuataz, 2002). (C) Isotherm SD and FD, and explanation difference in rehydration.

isotherm data of FD and SD lactose are almost identical at $a_w = 0.4$ as can be seen in Fig. 5C. Ultimately, if molecular mobility is high enough, the lactose will crystallize to the thermodynamic state of lowest free energy. The above suggests that the differences in the metastable thermodynamic state of the amorphous lactose can be diminished by conditioning of the molecular arrangements within the glassy state. Conditions would then have to be chosen such that molecular mobility is high enough for relaxation phenomena, while re-crystallization is avoided. For amorphous lactose the glass transition temperature at $a_w = 0.35$ (35% RH) is approximately 25 °C (Vuataz, 2002). Conditioning the powders at 35% RH at room temperature for not too long time would allow molecular rearrangements without re-crystallization. This experiment resulted in similar isotherms for both SD and FD lactose (Fig. 5A). Further research is needed to implement this preconditioning in a generic moisture sorption isotherm approach to analyze amorphous content in partially amorphous lactose samples regardless the history of the material.

5. Conclusions

Moisture sorption isotherms of blends of crystalline and amorphous lactose were determined. These isotherms were fitted to a BET formula and a linear relationship between a fit parameter (W_m) and the amorphous content was found. This makes it straightforward to assess the amorphous content of unknown samples with a high accuracy. It was shown that this accuracy could not be relied on in an approach that utilized the re-crystallization event of lactose. Unfortunately, the way of preparation of the amorphous lactose had a strong effect on the correlation factor of W_m and the amorphous content. A preconditioning step was introduced that made it possible to wipe out the history of the preparation. Both spray-dried and freeze-dried amorphous lactose gave the same moisture sorption isotherm. This will make it possible to obtain a new method for highly accurate determination of amorphous content, even at very low amounts.

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