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Quantifying the Degree of Disorder in Micronized Salbutamol Sulfate Using Moisture Sorption Analysis

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ABSTRACT Salbutamol sulfate is often micronized for use in dry powder inhalers. Therefore, it is of high interest to quantify the amorphous amount. It was investigated whether moisture sorption is able to measure the amorphous content of salbutamol sulfate. Different mixtures of amorphous and crystalline salbutamol sulfate were analyzed by moisture sorption. The amorphous material was obtained by spray drying. The measurement results were used to plot a calibration curve, which was used to quantify the amorphous amount in micronized salbutamol sulfate.

KEYWORDS Salbutamol sulfate, Dynamic vapor sorption, Moisture sorption, Crystallinity, Amorphicity, Milling

INTRODUCTION

Salbutamol sulfate is often used in dry powder inhalers, which makes it necessary to micronize the powder. Air jet milling may increase the degree of disorder in crystalline powders (Ward et al., 1995; Brodka-Pfeiffer et al., 2003). The amorphous areas are mainly located at the surface of the particles (Elamin et al., 1994). This might influence some characteristics of the substances, for example bioavailability and physical stability. Therefore, it is of high interest to determine the amorphous content of micronized salbutamol sulfate. It is also important to quantify the amorphous amount to be able to compare different batches. Brodka Pfeiffer et al. (2003) used isothermal microcalorimetry to quantify the amorphous amount of micronized salbutamol sulfate and suggested the use of moisture sorption analysis as another possibility quantifying the degree of disorder.

By powder X-ray diffraction (XRD) and differential scanning calorimetry (DSC) Saleki-Gerhardt et al. (1994) determined the amount of amorphous material with a quantification limit of 10% using sucrose as model compound. The quantification limit of amorphous lactose using isothermal microcalorimetry was 1% according to Briggner et al. (1994). Moisture sorption analysis is a powerful tool to quantify low amorphous amounts. So Buckton & Darcy (1995) were able to quantify the amorphous content of α-lactose monohydrate down to 0.125% by using moisture sorption analysis

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and Mackin et al. (2002) quantified the amorphous content of an experimental drug down to 0.2%. In this study, it was investigated whether the content of amorphous salbutamol sulfate could be quantified by this technique.

Moisture sorption is performed in humidity and temperature controlled chamber. A balance measures weight changes of the samples exposed to a defined humidity program. In comparison to crystalline materials, the amorphous state is characterized by a higher potential to absorb moisture. This leads to a higher mass increase of amorphous materials in comparison to crystalline materials, which is gravimetrically detectable. Furthermore the amorphous state is thermodynamically unstable and therefore, low molecular weight amorphous materials tend to recrystallize. Below the glass transition temperature (*T*g) recrystallization occurs spontaneously but slowly. When the temperature is raised above the *T*g the process proceeds faster. As absorbed water acts as a platicizer and lowers the *T*g, recrystallization can be accelerated by raising the relative humidity. Recrystallization is accompanied by loss of water and is associated with a mass decrease, which can also be gravimetrically determined (Buckton & Darcy, 1999). Both phenomena, the water uptake by absorption as well as the loss of water by recrystallization may be used to quantify the amount of amorphous material.

MATERIALS AND METHODS

Materials

Salbutamol sulfate was obtained from Glaxo Smith Kline UK Ltd., UK and STADA Arzneimittel AG, Germany.

Methods

Spray Drying

Amorphous powder was prepared by spray drying a solution of salbutamol sulfate (GlaxoSmithKline UK Ltd., UK) in water using a mini spray dryer B-191 (Büchi Labortechnik GmbH, Switzerland). A 10% (m/V) solution of salbutamol sulfate was spray dried at an inlet temperature of 150°C ± 5°C, an outlet temperature of 80°C ± 5°C, an air flow rate of 800 L/hr', an aspirator level of 90% and a pump speed set to 5 mL/min (Chawla et al., 1994). After spray drying, the amorphous salbutamol sulfate was stored in a desiccator containing silica gel until moisture sorption analysis was performed.

Differential Scanning Calorimetry (DSC)

Studies were perfomed using a DSC 821^e (Mettler-Toledo GmbH, Germany) with samples weighing approximately 2–3 mg, in aluminium pans with a pierced lid. The samples were heated from 0–350°C at a heating rate of 10.0°C/min and a nitrogen flow rate adjusted to 50 mL/min. Temperature and enthalpy were calibrated using indium.

Mixing

Different mixtures of amorphous and crystalline salbutamol sulfate were prepared in steps of 10% from 0% to 100% amorphous content with salbutamol sulphate of Glaxo. Mixtures between zero and 10% amorphous content were produced with salbutamol sulfate of STADA. Mixtures (approximately 2 g quantities) were prepared by mixing for 90 min in a glass vial using a TURBULA® shaker mixer (Willy A. Bachofen AG Maschinenfabrik, Switzerland). In order to keep the preparation conditions similar for all samples, the sample containing 100% crystalline salbutamol sulfate was treated in the mixer under the same conditions as the mixtures containing amorphous and crystalline salbutamol sulfate.

Moisture Sorption

Samples (approximately 2 g quantities) were examined by the moisture sorption system SPS11 (Projekt Messtechnik, Germany) using the following program. The relative humidity was first set to zero. In steps of 10% the relative humidity was raised to 90% and finally to 95%. Subsequently the relative humidity was decreased to zero RH the same way. This cycle was repeated once more. The equilibrium condition was set to 0.01% mass change per 60 min, which had to be reached before the program moved on to the next humidity step. The temperature was set to 25°C and the samples were weighed in time intervals of 6 min.

Milling

Crystalline salbutamol sulfate of Glaxo was milled in an air jet mill (50AS, Hosokawa Alpine AG, Germany). Injection pressure was set to 3.5 bar, milling pressure to 2.5 bar and feeding rate adjusted to approximately 1 g/min.

RESULTS & DISCUSSION

In the DSC trace of the amorphous sample, a melting peak was not recognizable. However, because of the detection limit of crystalline material in a mainly amorphous sample there might remain a certain inaccuracy. Mikhail & Turcotte (1997) were able to detect 0.1% crystalline material in an amorphous sample.

In Fig. 1 the moisture induced mass change of crystalline salbutamol sulfate over time is shown. At first a mass loss at 0% RH is detectable. This is caused by the loss of water, which has not been removed during storage over silica gel prior to analysis. Raising the relative humidity is associated with an increase of mass. The higher the humidity, the higher is the mass gain. Decreasing the relative humidity, after having reached 95% RH, leads to a decrease of mass. The mass change over time of the second cycle is equal to the first cycle. This shows that crystalline salbutamol sulfate has not undergone any changes during moisture sorption.

In Fig. 2 the mass change over time of the mixture containing 50% amorphous and 50% crystalline salbutamol sulfate is displayed. This sample shows a higher mass change than the crystalline sample at the same relative humidity. In contrast to the mass increase of the crystalline sample with increasing relative humidty, a decrease in mass is detectable at 60% RH, which is ascribed to recrystallization. At higher relative humidity the curves of the amorphous and the crystalline sample are similar. The mass change over time of the second cycle is comparable to that of the crystalline sample. With no RH recorded, it does not seem that the amorphous sample was completely dry at the end of this stage. This

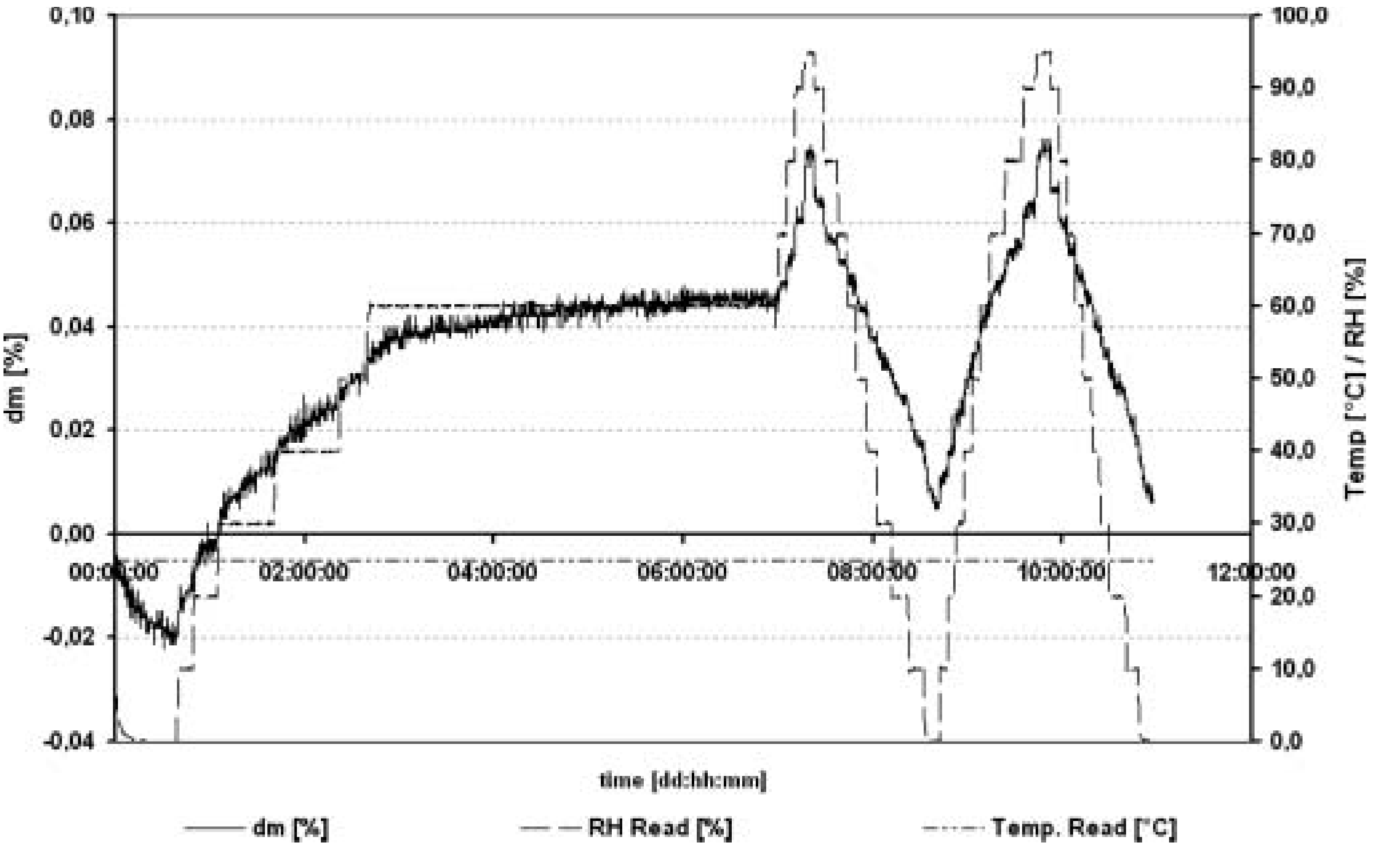

FIGURE 1 Mass Change Over Time of Crystalline Salbutamol Sulfate.

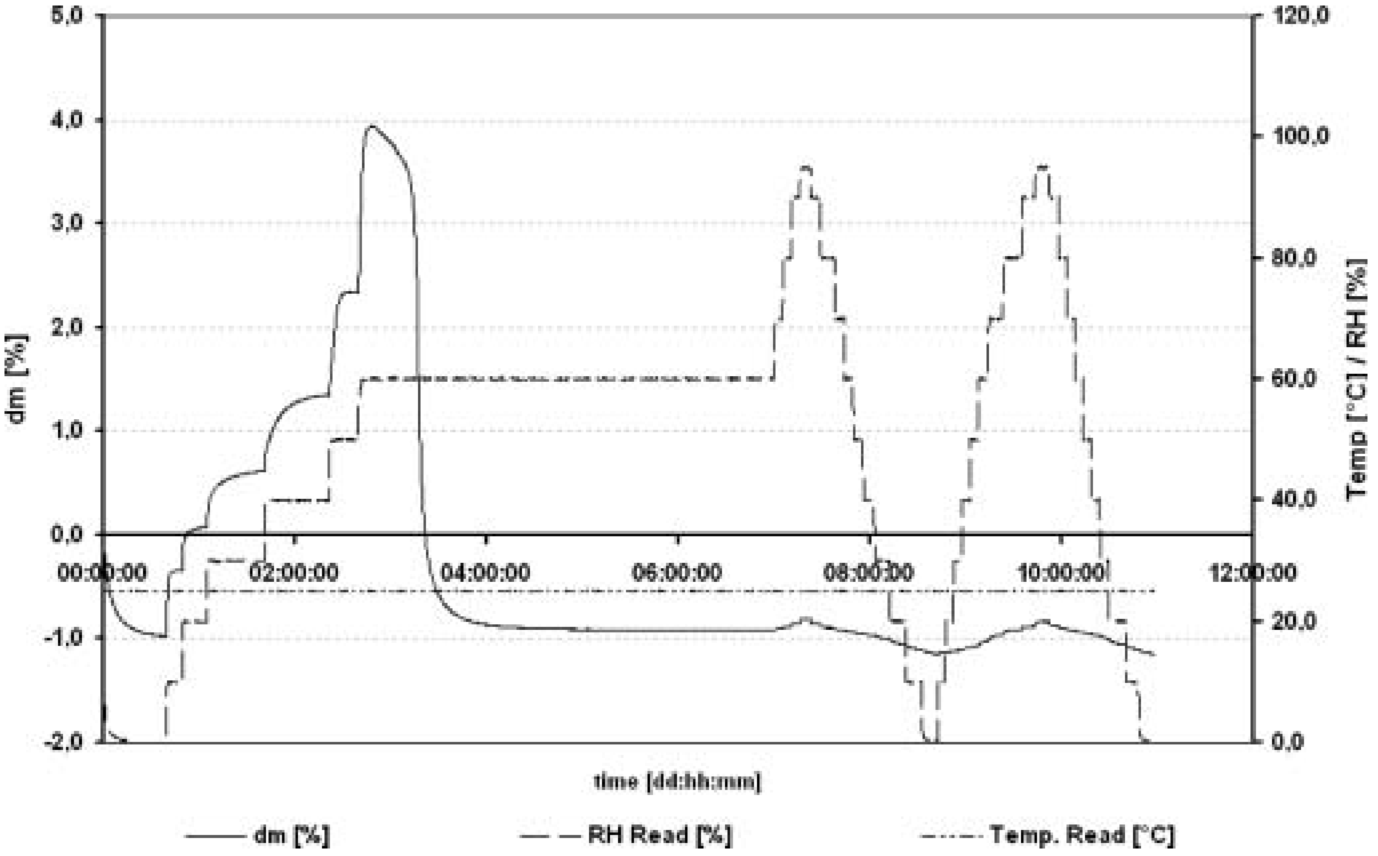

FIGURE 2 Mass Change Over Time of a Mixture Containing 50% Amorphous and 50% Crystalline Salbutamol Sulfate.

is because it takes a long time to desorb moisture from the bulk, so that our equilibrium conditions were insufficient at this point. Therefore, the data obtained at 0% RH was not used for quantification. The measurements at higher relative humidity show a plateau indicating equilibrium and, therefore, altering the equilibrium conditions seems not to be necessary.

Sorption isotherms are obtained by plotting the mass change at equilibrium against the relative humidity. The sorption isotherm of the 50% amorphous sample is shown in Fig. 3.

The sorption isotherms of mixtures containing 0, 20, 40, 60, 80, and 100% of amorphous amount are shown in Fig. 4, which were constructed using the data of the first sorption cycle. At 0% RH the mass change is standardized to be 0%, in order to be able to compare sorption isotherms of different samples. The higher the amorphous amount, the higher is the increase in mass during moisture sorption. Subsequently it was investigated, whether a linear relation between mass change and amorphous amount existed.

Different Methods to Generate a Calibration Curve

First, the mass change of the samples containing different amounts of amorphous material at a different relative humidity before recrystalization was plotted against the content of amorphous material (Fig. 5). In order to obtain a measure related to the amorphous content the mass change of the recrystallized sample (second cycle) has been subtracted from the mass change of the original sample (first cycle) at a given relative humidity, a method also used by Mackin et al. (2002).

Second, it is possible to use the area between the first and the second sorption cycle ("ABC") to quantify the amorphous amount (Fig. 6). Table 1 displays the equations of the respective regression lines of the calibration curves. All calibration curves deliver acceptable regression lines, but 10 and 20% RH are probably stronger influenced by an error of weight, because of lower mass changes.

Third, the mass loss induced by recrystallization could be used in order to quantify the amorphous

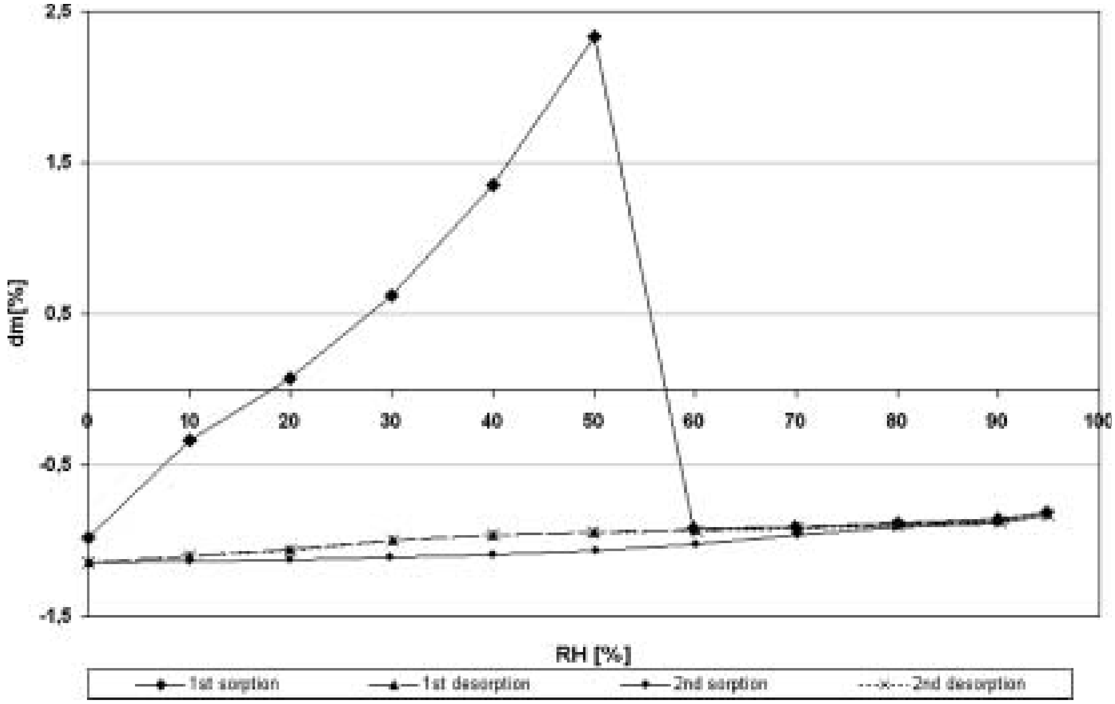

FIGURE 3 Sorption Isotherm of a Mixture Containing 50% Amorphous and 50% Crystalline Salbutamol Sulfate.

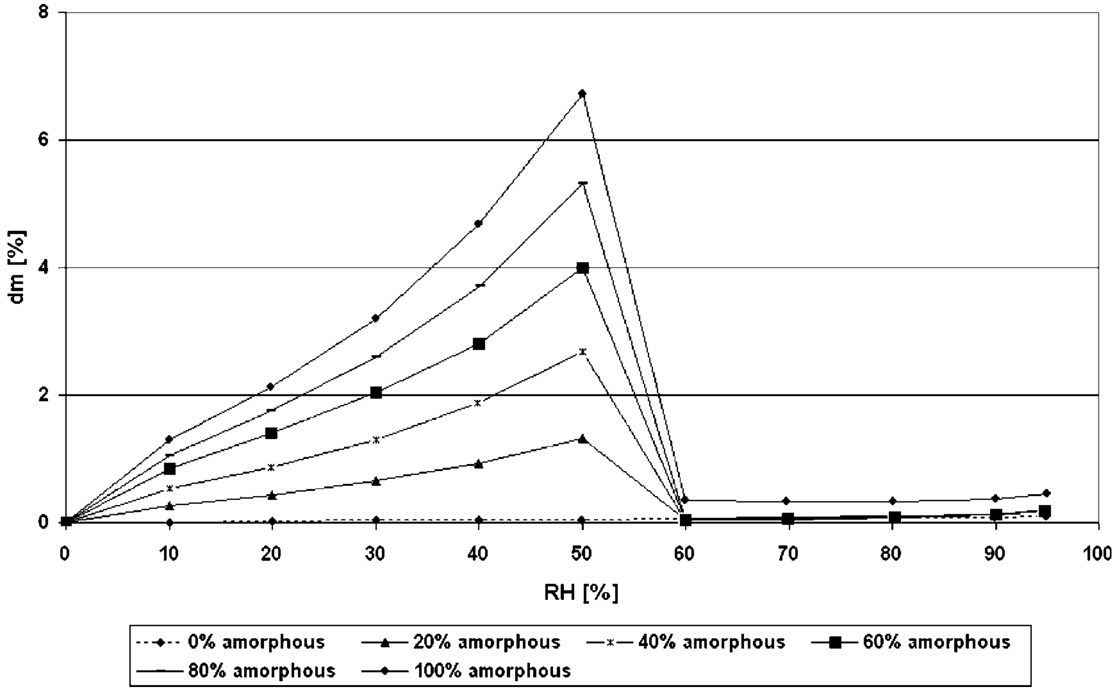

FIGURE 4 Sorption Isotherms of Samples with Different Amorphous Contents Using the Data of the First Sorption Cycle.

content. It might be calculated by subtracting the weight of the sample after recrystallization at 60% RH from the weight before recrystallization at 50% RH. However, there is an inaccuracy arising in doing so as the crystalline material contained in the sample before recrystallization undergoes a weight increase when the relative humidity is raised. Therefore, the subtracted value (at 60% RH) is too high. Accordingly, this

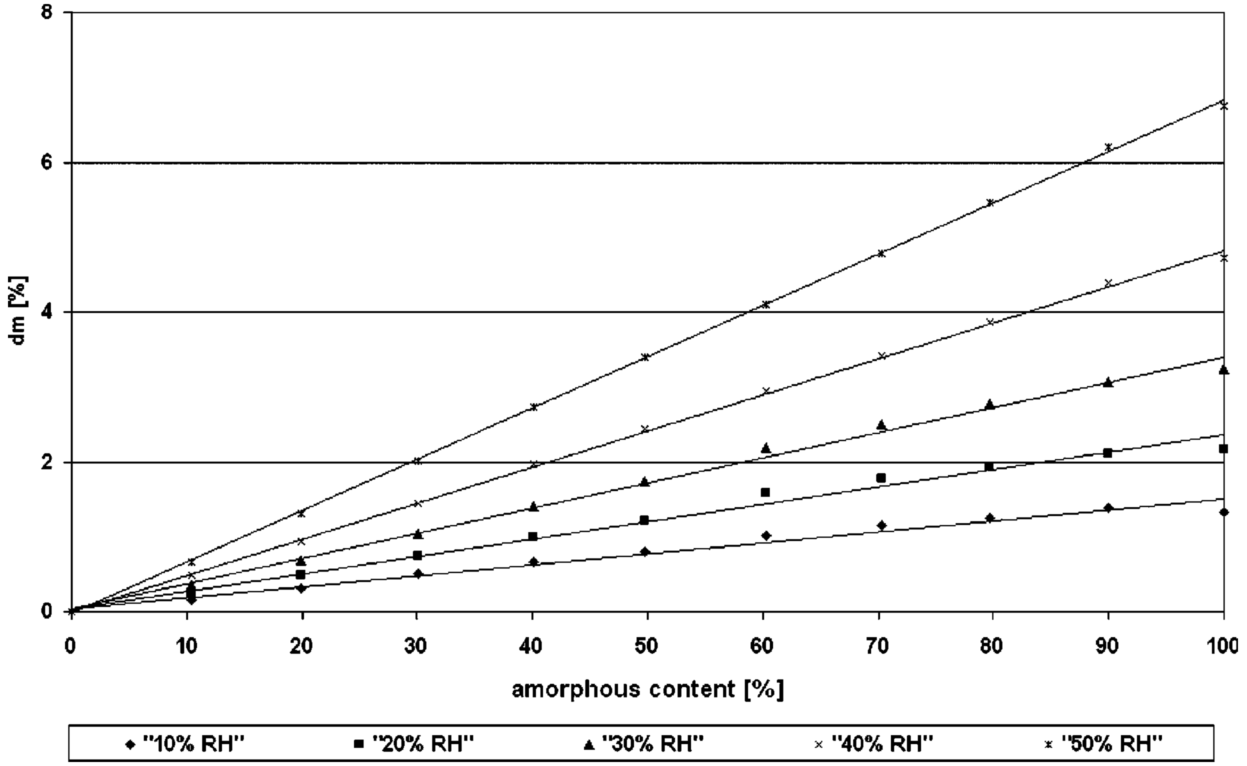

FIGURE 5 Calibration Curves Obtained Using the Difference Between the Mass Change of the Original Sample (First Sorption Cycle) and the Recrystallized Sample (Second Sorption Cycle) at 10, 20, 30, 40 and 50% Relative Humidity.

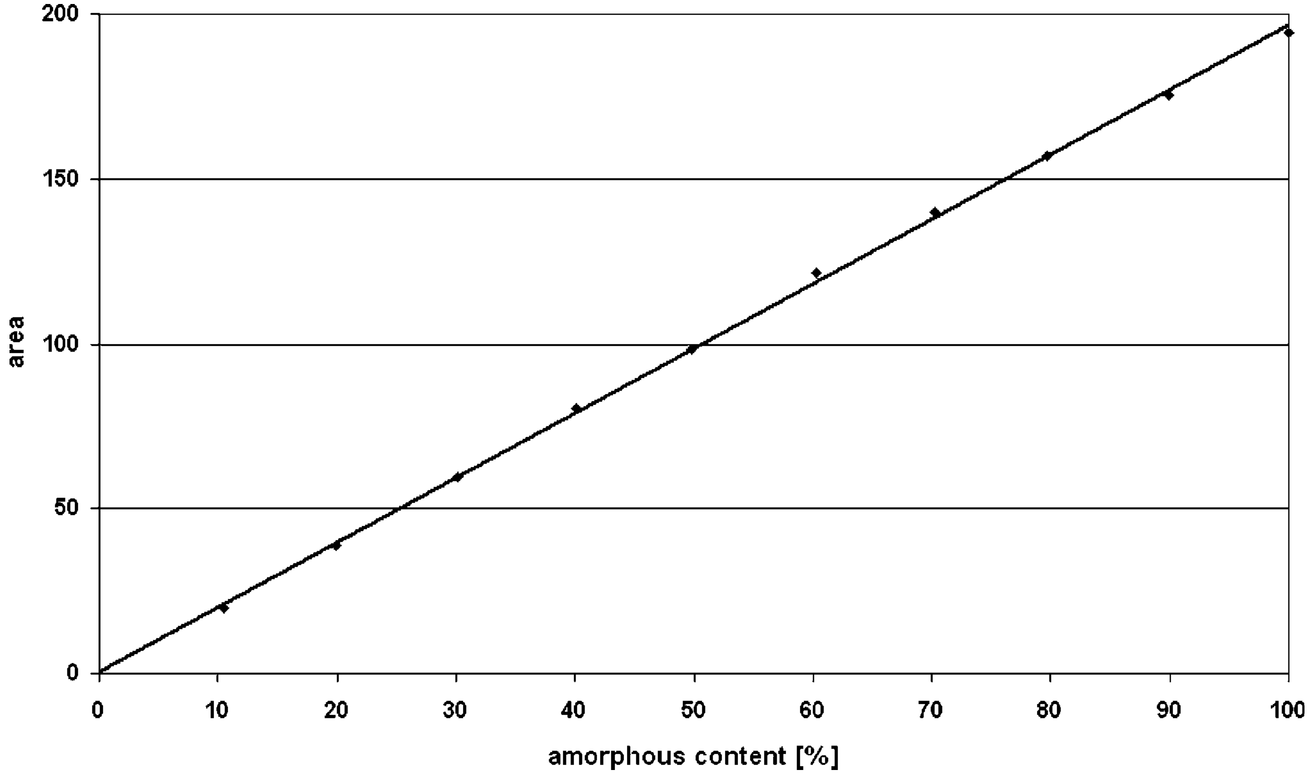

FIGURE 6 Calibration Curve Obtained Uusing the Area Between the First and Second Sorption Cycles.

TABLE 1 **Equations of Regression Lines of the Calibration Curves From Fig. 5**

Calibration curve	Equation	R^2
10% RH	y = 0,0146x + 0,043	0,9782
20% RH	y = 0,0232x + 0,0406	0,9873
30% RH	y = 0,0337x + 0,0309	0,9955
40% RH	y = 0,0482x + 0,0059	0,9993
50% RH	y = 0,0686x – 0,0279	0,9997
"ABC"	y = 1,9614x + 0,3361	0,9995

method of quantification seems to be arbitrary and thus not used for the determination of the amorphous content.

Quantifying the Amorphous Content of Air Jet Milled Salbutamol Sulfate

The moisture induced weight change of milled products differ slightly from mixtures used in establishing the calibration curves (Fig. 7). Recrystallization starts at 40% RH, whereas the samples of the calibration curve recrystallize at 60% RH. This may be mostly due to surface amorphous material in the micronized sample in comparison to bulk amorphous material in the spray dried products. The recrystallization is not accomplished in one interval, but covers some more steps at higher relative humidity. Additional investigations will be necessary to explain this behavior. The corresponding sorption isotherm is shown in Fig. 8.

Table 2 displays the amorphous amount of the milled sample obtained by using different calibration curves. As the recrystallization behavior of the air jet milled salbutamol sulfate differs from spray dried products, the calibration curves for 40% RH and 50% RH are not used to measure the amorphous content, because the recrystallization has already started before reaching 40% RH. To minimize the error of weighing the calibration curve at a relative humidity, which is as high as possible, seems to be reasonable. Therefore, using the calibration curve at 30% RH turned out to be the most reliable method for quantifying the amorphous content. According to this method the amount of amorphous salbutamol sulfate is 6.4%.

The determination of the amount of amorphous material using the "ABC" leads to the highest value.

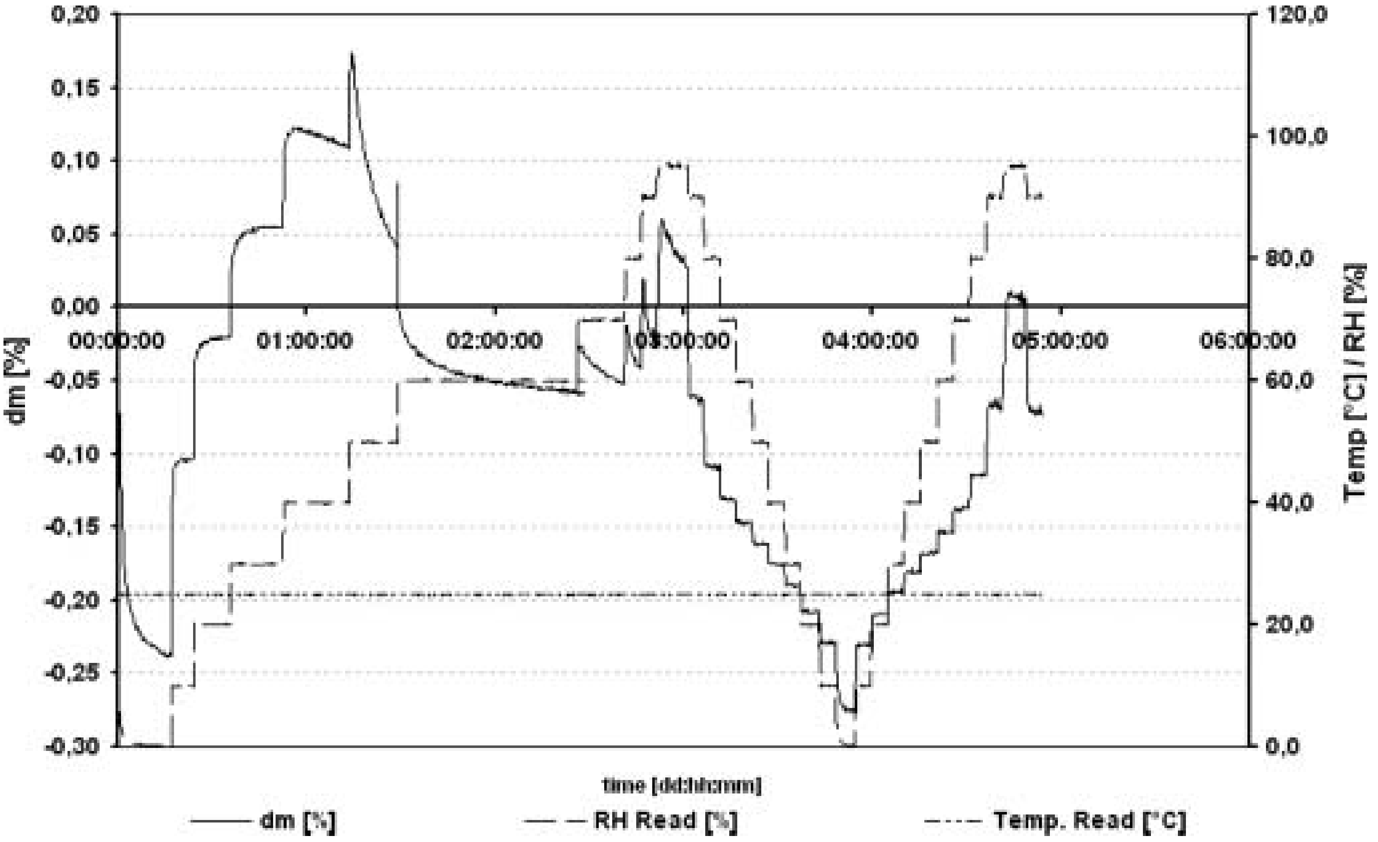

FIGURE 7 **Mass Change Over Time of Micronized Salbutamol Sulfate.**

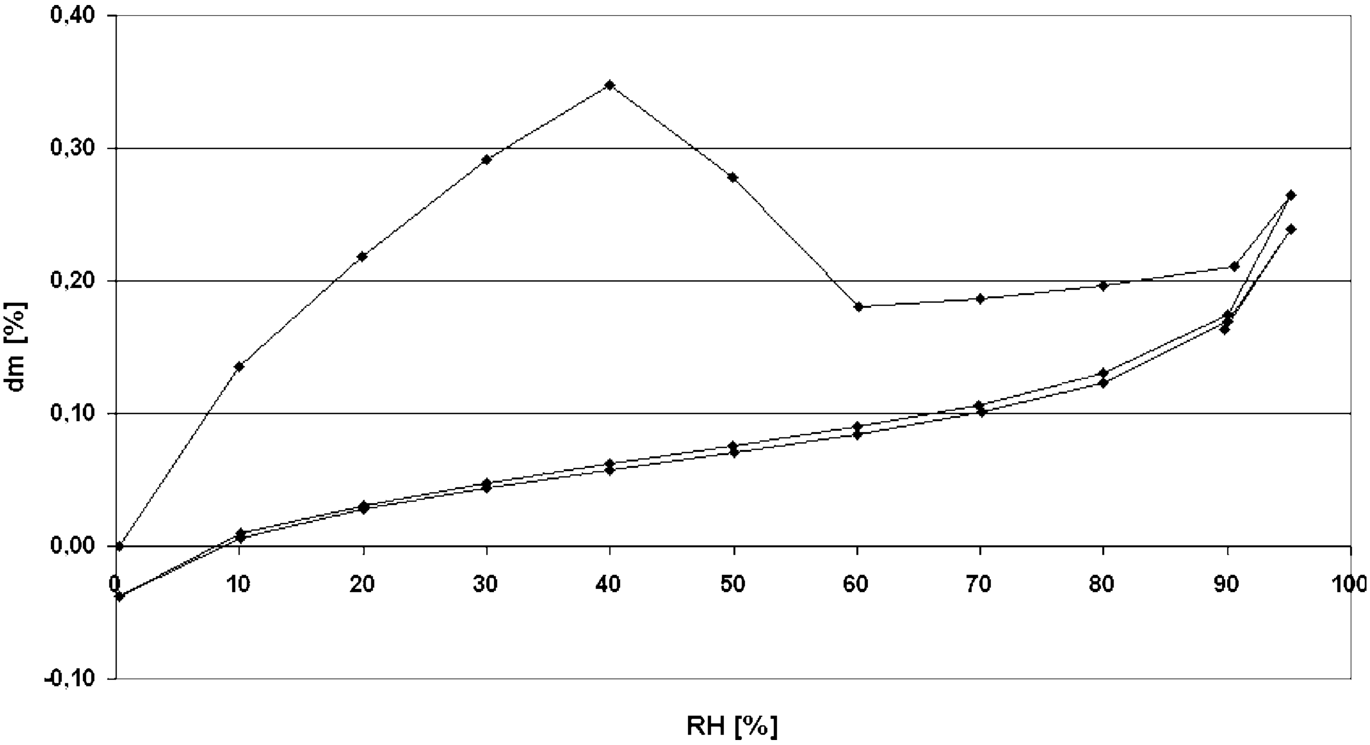

FIGURE 8 Sorption Isotherm of Micronized Salbutamol Sulfate.

TABLE 2 Calculated Amorphous Amounts by Using Different Calibration Curves

Method	Amorphous amount (%)
10% RH	5.9
20% RH	6.4
30% RH	6.4
"ABC"	6.8

This might be due to recrystallization covering several humidity steps. Furthermore the shape of the sorption isotherm of the air jet milled sample is not equal to the crystalline sample for a relative humidity higher than 60%. These factors may induce some inaccuracies rendering this method of determination not very suitable.

As the measured amorphous amount of the milled sample was 6,4% as determined by using the calibration curve at 30% RH and as the measured amorphous amount of the milled sample was lower in comparison to the sample with the smallest amorphous content used for the calibration curve, it was investigated whether the calibration curve shows linearity between zero and 10% amorphous content, as well. Therefore, samples with an amorphous amount of 0.5, 1, 5, and 10% were measured. Linearity between the amorphous content and the mass changes can be seen in Fig. 9.

CONCLUSION

Vapor sorption is a valuable tool to determine the amorphous content of salbutamol sulfate. There are several methods to generate calibration curves, which might be used for the quantification of the amorphous amount, therefore, the method is to be selected carefully. In this study it has been concluded, that the quantification of the amount of amorphous material in micronized salbutamol sulfate is most reliably determined by measuring the difference in the mass change before and after recrystallization at 30% RH. The most accurate results are expected to be obtained using higher relative humidity because the differences in mass change increases with increasing RH, however, it has been found that using higher relative humidity may result in an inaccurate determination of the amorphous amount of the micronized product. This is because the mass change before recrystallization of the micronized sample can not be determined reliably at relative humidity of 40% and above. This was seen when recrystallisation of the milled sample occurred at 40% RH. This is in contrast to the spray-dried material, which has been used to generate the calibration curves recrystallizing at 60% RH.

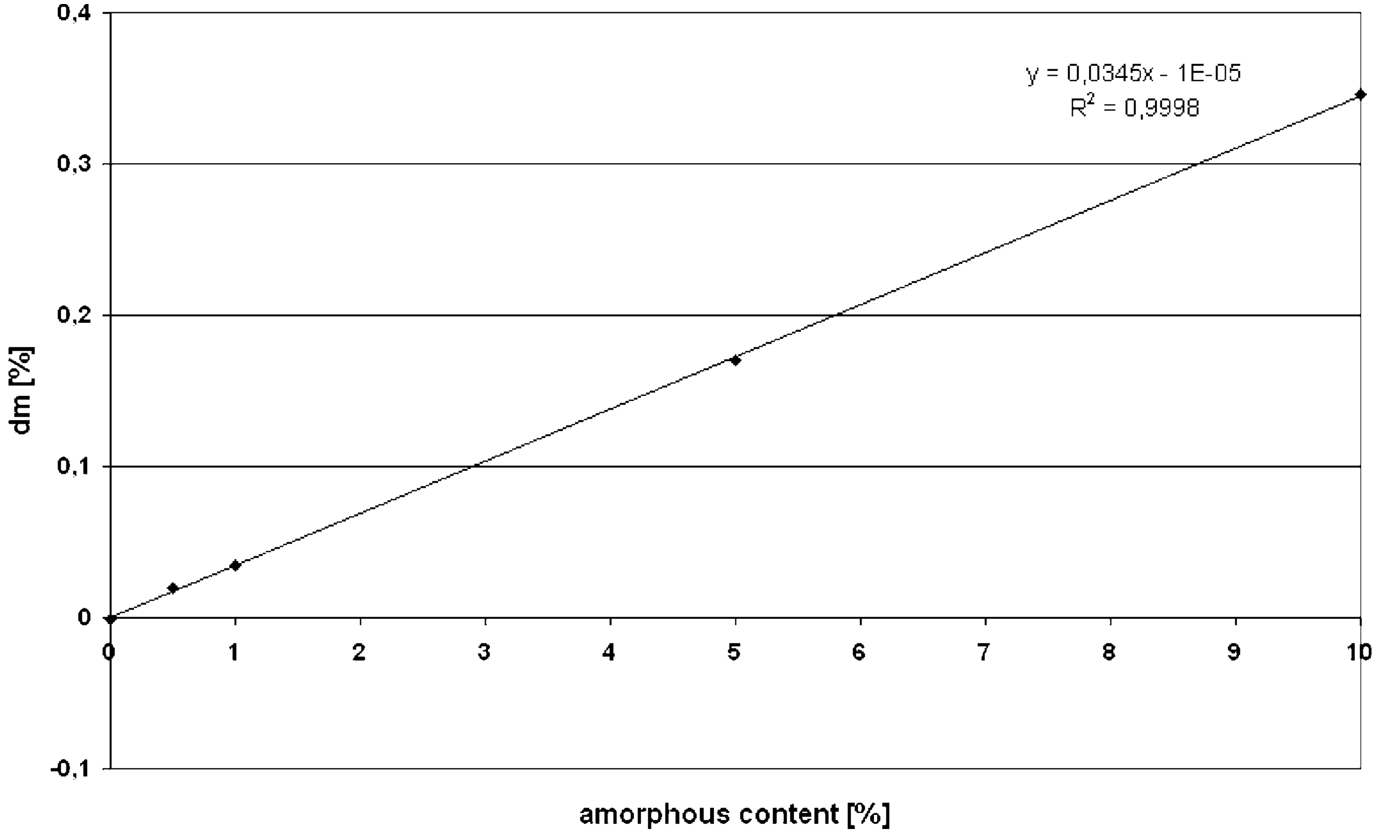

FIGURE 9 **Calibration Curve for Amorphous Contents Lower Than 10% Obtained After Using the Difference Between the Mass Change of the Original Sample (First Sorption Cycle) and the Recrystallized Sample (Second Sorption Cycle) at 30% Relative Humidity.**

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