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Research Paper

Applicability of the one-step DVS method for the determination of amorphous amounts for further different hydrophilic and hydrophobic drugs

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A B S T R A C T

In a former publication the authors showed that low amounts of amorphous content (LOQ of 0.5%) in a hydrophobic model API (Ciclesonide) can be measured with an individually adjusted one-step dynamic organic vapor sorption (DVS). In this investigation the applicability is tested on various APIs which differ in lipophilicity (poor water solubility) and hygroscopicity (absorption of water).

The vapor sorption method proved to be applicable in almost all cases. Moisture sorption isotherms were determined for all five investigated crystalline and amorphous APIs. However, it was necessary to select the parameters individually for each API. The used solvents (water, methanol, isopropanol and methylene chloride) and the humidity-levels (0.05 p/p_0 until 0.5 p/p_0) were chosen carefully because otherwise the amorphous amounts switch to their crystalline counterparts and are not detectable. The production of fully amorphous samples (absence of crystalline material measured by DSC, mDSC and XRPD) was optimized over several trials. As successfully methods proved ball-milling, freeze-drying, spray-drying and/or quench cooling. In the next step these fully amorphous amounts were blended with crystalline starting material to calibration curves (Turbula blender, influence of electrostatic charge to homogeneity) for the calculation of amorphous content.

In summary, the following presented methods were used to determine and quantify low amorphous amounts (between 1.5% and 17.0%) in jet-milled powders (grinding pressure of 8 bar, 1–3 grinding cycles), respectively.

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1. Introduction

In the last years the interest in amorphous regions grew more and more. The production techniques, the detection methods, solubility tests and stabilization trials are in the focus and require greater attention [1–4]. The common active pharmaceutical ingredients (APIs) are synthesized as crystalline solids and processed by standard pharmaceutical operations. These processing steps such as milling [5], blending [6] and even sieving may lead to structural changes, crystal defects and amorphous regions. The above named disorders may have a huge potential to change during handling and storage and should be limited to a minimal level [7]. This

metastable state is prone to change over time, initiated by changes in relative humidity and/or temperature [8] and may induce re-crystallization [9] of the amorphous regions and particle size changes post-production.

In a former publication the authors showed that low amorphous content in a hydrophobic model API (Ciclesonide) can be measured with an individually adjusted one-step dynamic organic vapor sorption (DVS). This gravimetric method quantifies exactly the amorphous content once a suitable solvent, the correct p/p_0 value and the exact temperature have been found. After the sample went through a drying phase, the moisture is adjusted with the particular solvent (one-step). As soon as the equilibration of mass with definite humidity is balanced, different amorphous amounts can be calculated on the basis of different absorption values. Generally, the background adsorption of crystalline starting material and the absorption of blends with known amorphous content

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(between 0.5% and 15.0%) are determined, respectively. Finally, the amorphous amounts of unidentified samples are measured and calculated using the calibration curve [10].

Over the years, only a few approaches focused on organic solvents used in DVS measurements [11,12]. For example Mackin et al. used acetone as a solvent and Samra et al. used mixtures of ethanol/water and ethanol/n-propanol in their investigations with hydrophobic substances. Mostly different analytical techniques were used such as X-ray Powder Diffraction (XRPD), Differential Scanning Calorimetry (DSC), Isothermal Microcalorimetry or Near infra-red Spectroscopy [13–15].

In the following study the possibility of applying this described DVS method (based on equilibrium moisture uptake) is shown and even extended to different hydrophilic and hydrophobic active pharmaceutical ingredients (APIs). These physico-chemical characterized APIs differ in lipophilicity and hygroscopicity. Therefore different production parameters are required to produce fully amorphous amounts (e.g. ball-milling, freeze drying, spray drying and quench cooling). These fully amorphous samples were blended with crystalline starting material in order to generate calibration curves (Turbula blender). The absence of crystalline material in the fully amorphous samples is measured by DSC, mDSC and XRPD. Furthermore, different polar and nonpolar solvents (water, methanol, isopropanol and methylene chloride) and variable humidity-levels (between $0.05\ p/p_0$ and $0.5\ p/p_0$) are required to test screening measurements. Finally with optimized parameters the calculation of amorphous parts in micronized powders (jet-mill: grinding pressure of 8 bar and 1–3 grinding cycles) is feasible by these one-step DVS methods (1.5% up to 17.0%). In particular, the screening process of hydrophobic substances is difficult and time consuming. Thereby, it is necessary that the parameters are selected individually for each API. It must be kept in mind that the use of only certain solvents (toxicity in terms of personal safety, as well as device compatibility) is possible. This work is intended to show a possible approach on the procedure of adapting and refining the method for new APIs in the future. The primary objective is to develop a fast (less than 2 days) and accurate method with high selectivity, respectively. A general guidance for the adaptation of the method to other drugs is established.

2. Materials and methods

2.1. Materials

Crystalline Ipratropium bromide monohydrate (ITB, particle size $d_{50} = 1.8 \pm 0.0\ \mu m$), crystalline Formoterol fumarate (FF, particle size $d_{50} = 1.6 \pm 0.0\ \mu m$) and crystalline Salbutamol sulfate (SBS, particle size $d_{50} = 9.1 \pm 0.1\ \mu m$) are chosen as hydrophilic model drugs. Crystalline Glibenclamide (GC, particle size $d_{50} = 34.5 \pm 0.4\ \mu m$), crystalline Budesonide (BS, particle size $d_{50} = 1.7 \pm 0.0\ \mu m$) and crystalline Ciclesonide (CS, particle size $d_{50} = 57.9 \pm 3.0\ \mu m$) represent the hydrophobic model drugs. The water used was of double distilled (ddH$_2$O) quality (GEA Diessel GmbH, Hildesheim, Germany), methanol was supplied by J.T. Baker (Deventer, The Netherlands), isopropanol for the DVS measurement was supplied by AppliChem GmbH (Darmstadt, Germany) and methylene chloride was supplied by LGC Standards GmbH (Wesel, Germany).

2.2. Methods

2.2.1. Ball-milling (BM)

Crystalline APIs were ball-milled in zirconium oxide grinding jars (500 ml) containing a defined number of zirconium oxide grinding balls (30 mm) using a Retsch PM 100 mill (Haan,

Germany). The grinding time, the rotation speed and the filling level were adjusted for each API, respectively. During the process the temperature (cold storage room: 1–3 °C) was monitored. The amorphous samples (ball-milled powders) were stored over P$_2$O$_5$ in a desiccator (volume 2.4 l, room temperature) to avoid re-crystallization. In the following these fully amorphous powders were used for the preparation of blends for the calibration curves.

2.2.2. Freeze-drying (FD)

Amorphous ITB and SBS were prepared by freeze-drying of a 2% (w/w) aqueous solution with a Christ alpha 1–4 (Osterode, Germany). First the fluid was applied by means of a burette in liquid nitrogen (Dewar vessel, 50 ml); the frozen balls were sieved and frozen at −30 °C in the freeze dryer for 15 min. Afterward vacuum was applied (2.56 mbar) and the primary drying process was started at −10 °C and lasted 40 h. Then the secondary drying at 0.0001 mbar was started at +10 °C and lasted 20 h.

The amorphous samples (freeze-dried powders) were stored over P$_2$O$_5$ in a desiccator (volume 2.4 l, room temperature) to avoid re-crystallization. In the following these powders were used for the preparation of blends.

2.2.3. Spray drying (SD)

Amorphous SBS was produced by spray-drying of a 5% (w/w) aqueous solution with a Büchi Mini Spray Dryer B-290 (Flawil, Switzerland) at an inlet air temperature of 150–151 °C, an outlet temperature of 80–82 °C, feed flow of 4.5 ml/min and an aspiration of 100%.

Amorphous BS was prepared by spray drying of a 5% (w/w) methylene chloride solution at an inlet air temperature of 50–51 °C, an outlet temperature of 34–35 °C, feed flow of 3.75 ml/min and an aspiration of 100%. The amorphous samples (spray-dried powders) were stored over P$_2$O$_5$ in a desiccator (volume 2.4 l, room temperature) to avoid re-crystallization. In the following these powders were used for the preparation of blends.

2.2.4. Quench cooling (QC)

1 g of GC was weighed into an aluminum cup and melted on a Kofler-hot bench (Wagner Munz, Munich, Germany) at 220 °C. The melt was carefully transferred into liquid nitrogen. The amorphous powder was sieved (425 μm) and to avoid re-crystallization stored over P$_2$O$_5$ (room temperature). In the following this powder was used for the preparation of blends.

2.2.5. Jet-milling (JM)

5 g of each crystalline API was jet-milled (JM1) using the Jet-O-Mizer Model 00 (Fluid Energy Aljet, Plumsteadville, USA). The grinding pressure was adjusted to 8.0 bar and three grinding cycles (Gc 1–3) were performed. To avoid re-crystallization the amorphous samples were stored over P$_2$O$_5$ (room temperature). In the following these powders were used for the development of a DVS method and as test samples for calculating unknown amorphous content.

2.2.6. Blending calibration curve (CC)

Firstly, powder blends with amorphous ball-milled or freeze-dried API (0.25%, 2.0%, 5.0%, 8.0% and 15.0%) and crystalline API were sieved (mesh size: 500 μm) and then accurately weighed into a stainless steel container by using the double sandwich method. All blends were prepared with a Turbula mixer (W.A. Bachofen AG, Basel, Switzerland). The mixing speed (42 rpm) and the mixing time (3 × 15 min and after every 15 min sieved with the 500 μm sieve) did retained unchanged. For micronized, crystalline starting materials (FF/BS) an electrostatics discharge stick (Haug GmbH, Leinfelden, Germany) had to be used in order to guarantee homogeneous mixtures. In the following these blends

were used to calculate the amorphous amounts by DVS measurements.

2.2.7. Particle size distribution (PSD)

The particle size distribution (PSD) of the micronized material was determined by the Sympatec Helium–Neon Laser Optical System (HELOS, Sympatec GmbH, Clausthal-Zellerfeld, Germany). The powders were dispersed by compressed air (3 bar) into the measuring zone. The calculation of the particle diameter was done with Windox 5 software based on FREE (Fraunhofer Enhanced Evaluation).

2.2.8. Scanning electron microscopy (SEM)

Surface morphologies were investigated by using a scanning electron microscopy (SEM). The samples were prepared by fixing the powder on a carbon sticker and coating the sample with gold using a BAL-TEC SCP 050 Sputter Coater (Leica Instruments, Wetzlar, Germany). The samples were then visualized with a Zeiss Ultra 55 plus (Carl Zeiss NTS GmbH, Oberkochen, Deutschland) using the SE-2 detector and a working voltage of 2 kV.

2.2.9. X-ray powder diffraction (XRPD)

All powders were examined by X-ray powder diffraction (XRPD, Stoe&Cie GmbH, Darmstadt, Germany). The X-ray Cu anode was operated at a voltage of 40 kV and strength of 30 mA. The samples were measured in the range of 8–35° at a step rate of $2\theta = 0.05°$ with 2 s measuring time per step. This technique was used for the identification of the fully amorphous state.

2.2.10. Differential scanning calorimetry (DSC)

In order to determine the re-crystallization temperature (T_c) and the melting point (T_m) the samples were analyzed by differential scanning calorimetry (Perkin Elmer PYRIS Diamond DSC, Massachusetts, USA). For the measurement 2–3 mg powder was accurately weighed into pierced aluminum DSC pans. The samples were run at a heating rate of 10 °C min^{-1} under a nitrogen flow of 20 ml min^{-1}. A temperature range from 30 °C to 300 °C was used.

2.2.11. Modulated differential scanning calorimetry (mDSC)

A modulated temperature DSC (Perkin Elmer PYRIS Diamond DSC, Massachusetts, USA) was used during the study to determine the change in heat capacity and to measure the glass transition temperature (T_g) of the samples. 5–6 mg powder was filled into pierced aluminum DSC pans. All samples were measured at a heating rate of 5 °C min^{-1}, step rate 3 °C, isothermal 1 min and between a temperature range from 0 °C to 150 °C (50 single steps). This technique was used for a fast identification of the amorphous state.

2.2.12. Inverse gas chromatography (iGC)

The dispersive surface energy was determined by means of inverse gas chromatography (Surface Measurement Systems Ltd., SMS, London, UK). At least 100 mg powder was packed into pre-silanized glass column, with an inner diameter of 3 mm and length of 30 cm, with silanized glass wool. The columns were compacted with the iGC column packer (SMS, London, UK) at a medium intensity (corresponds to level 6) for 10 min and conditioned for 2 h at 0% RH and 303 K to remove impurities and physisorbed water. Different alkanes (decane, nonane, octane, heptane and hexane) were injected three times. The Flame Ionization Detector was used to measure the highest surface energy of the samples. The dispersive part of the surface energy was calculated by using the iGC software (SMS, London, UK) for all starting materials of each API.

2.2.13. Determination of residual moisture – IR scale

The moisture analyzer (Sartorius MA 45, Sartorius Weighing Technology GmbH, Göttingen, Germany) was used to determine the residual moisture of hydrophilic and hydrophobic APIs. At least 1.8 g was weighed and dried to constant mass at 105 °C. Thereafter the water loss was calculated based on the initial mass.

2.2.14. Density measurement

The true density was measured with a Helium pycnometer (Pycnomatic ATC, Porotec, Hofheim, Germany). A suitable amount of powder was accurately weighed into the vessel, followed by a ten-fold determination. Every powder was analyzed three times.

2.2.15. Dynamic vapor sorption (DVS)

Screening vapor sorption measurements were taken (DVS-1, Surface Measurement Systems Ltd., London, UK) using a gravimetric method to determine the humidity-dependent increase of mass (1 sample). For all screening measurements water was used as a solvent to determine moisture sorption isotherms of crystalline starting materials and fully amorphous samples. Furthermore the contrasting increase of mass between hydrophilic and hydrophobic APIs was investigated. For all measurements the p/p_0 value was increased from 0.0 p/p_0 to 0.9 p/p_0 (steps 0.1 p/p_0) and then decreased to 0.0 p/p_0 (steps 0.1 p/p_0) and finally the cycle was repeated a second time (Table 1). The equilibrium criteria for each step was reached after no further mass change was detected within four single mass data points.

Further dynamic vapor sorption measurements were taken (DVS-HT, Surface Measurement Systems Ltd., London, UK) using a gravimetric method to determine the humidity-dependent increase of mass (10 samples) and to classify amorphous content after a calibration. For each hydrophilic and hydrophobic API

Table 1
DVS screening process for all five APIs: Choice of solvent, p/p_0 value and amorphous phase.

Samples	Screening			Development of the one-step DVS-method		
	Phase	Solvent	DVS-1	Phase	Solvent	DVS-HT
ITB	Fully amorphous Fully crystalline	ddH$_2$O	0.0–0.9 p/p_0	Semi-crystalline	ddH$_2$O	0.1 and 0.2 p/p_0
FF	Fully amorphous Fully crystalline	ddH$_2$O	0.0–0.9 p/p_0	Semi-crystalline	ddH$_2$O	0.3 and 0.4 p/p_0
SBS	Fully amorphous Fully crystalline	ddH$_2$O	0.0–0.9 p/p_0	Semi-crystalline	ddH$_2$O	0.3 and 0.4 p/p_0
BS	Fully amorphous Fully crystalline	ddH$_2$O	0.0–0.9 p/p_0	Semi-crystalline	Methanol Isopropanol Methylene chloride	0.05 and 0.1 p/p_0 0.05 and 0.1 p/p_0 0.05 and 0.1 p/p_0
GC	Fully amorphous Fully crystalline	ddH$_2$O	0.0–0.9 p/p_0	Semi-crystalline	ddH$_2$O Methanol Isopropanol Methylene chloride	0.3 and 0.5 p/p_0 0.05 and 0.1 p/p_0 0.05 and 0.1 p/p_0 0.05 and 0.1 p/p_0

individual parameters were used. Different solvents (polar and nonpolar) and different p/p_0 values (0.05–0.5 p/p_0) were adjusted for screening measurements, respectively (Table 1). Hydrophilic APIs were measured with ddH$_2$O and hydrophobic APIs were screened with water, methanol, isopropanol and methylene chloride.

2.2.16. Conditioning of the APIs

To avoid re-crystallization the amorphous samples (micronized powders and blends) were stored over P$_2$O$_5$ in a desiccator. In order to investigate the surface of the hydrophobic samples and the interaction with organic solvent, these samples were stored over 10 ml methanol or isopropanol (BS), isopropanol (CS) and isopropanol or methylene chloride (GC). The samples were compared by SEM analysis after 2 h of storage in a desiccator (volume: 2.4 l, room temperature).

3. Results

3.1. Preparation of fully amorphous samples

Not all preparation techniques proved themselves to be suitable for each API. The ball-milling method was successfully applied to 4 of 5 new tested APIs for the preparation of fully amorphous content. Table 2 shows the final and optimized parameters for the ball-milling process of FF, SBS, BS and GC. Regarding the hygroscopic drug ITB the ball-milling process was not suitable. Even after the grinding chamber was fumigated with argon and the starting material was dehydrated (was lowered from 3.7% to 0.4% water content, 2 h at 150 °C) it was not possible to receive a fully amorphous ITB. Therefore other production methods were required such as freeze-drying.

In this investigation the freeze drying process was tested only with the hydrophilic, water-soluble substances, and proved to be the medium of choice especially for ITB and SBS. One disadvantage is the wide particle size distribution for future preparations of homogeneous mixtures for the calibration curve. This method was not applied on organic soluble substances due to the incompatibility of the freeze-dryer. The spray-drying process was successfully performed with SBS and BS. ITB showed a fast recrystallization which leads to a crystalline powder [16]. For certain parameters a clear melt was found in the cyclone of the spray-dryer which can be connected to the hygroscopic behavior of the API. For GC a low solubility was determined for common organic solvents such as methanol, isopropanol and methylene chloride. Spray-drying from a suspension led to crystalline powders. As an alternative method to the spray- and freeze-drying processes the quench cooling was used for the preparation of amorphous GC [17], other APIs (e.g. ITB and FF) showed decomposition at the melting point and therefore impracticability for this approach. It should be emphasized that structural and thermal properties and characteristics may differ slightly once different preparation techniques are used [18].

Table 2
Parameters for all used APIs in the ball-milling process for the production of fully amorphous samples.

Samples	Grinding time (h)	Rotation speed (U/min)	Number of grinding balls	Filling level (g)
FF	16	340	4	2.5
SBS	24	450	6	25.0
BS	20	345	2	2.0
GC	24	240	3	2.0

3.2. Characterization of physico-chemical properties (DSC, mDSC and XRPD)

The following DSC and mDSC measurements ought to give a short overview of the characteristic properties of the crystalline starting material as well as they confirm the amorphous samples that have been produced by the foresaid methods. In this investigation endothermic melting events for crystalline samples and exothermic re-crystallization events for amorphous products are determined by DSC. The mDSC method was used for the determination of the glass transition (T_g) of amorphous samples (Table 3).

It was also possible to correlate the glass transition of each API with its molecular weight. As expected, an increase of the glass transition temperature can be linked to an increasing molecular weight. Higher molecular weight leads to lower mobility [19]. In this case the spray-dried Budesonide shows an exception. Generally, with an increasing T_g a slower re-crystallization behavior is expected and might lead to a higher stability of amorphous amounts. Significant differences between hydrophilic and hydrophobic samples could not be detected.

Nevertheless, XRPD-patterns of crystalline and amorphous samples were determined. For all APIs the crystalline starting material showed sharp peaks in contrast to the amorphous samples (BM/FD), which produce a broad background signal (Fig. 1). These results confirmed the absence of crystalline material in the fully amorphous samples and supported the findings from the DSC and the mDSC analytics. In the following these amorphous samples were used to produce the calibration curves (0.25–15.0%) for the calculation of the amorphous content of micronized powders. Only ITB was produced with a freeze dryer (Fig. 1, ITB*), while in this case all other samples were prepared with the described ball-mill.

### 3.3. Characterization and determination of the amorphous content by DVS

As expected, screening vapor sorption measurements (DVS-1) showed different results for crystalline hydrophilic and hydrophobic APIs (Fig. 2A and B). Moisture sorption isotherms of crystalline ITB showed the highest mass adsorption (1.1% change in mass) at 0.9 p/p_0 of all six investigated APIs (Fig. 2A). This mass increase of ITB was followed by the crystalline FF (0.6%) and SBS (0.4%). These hydrophilic, crystalline materials adsorb water on the surface [20] and show a high affinity to the polar solvent. In this case the bromide has the greatest affinity to water vapor compared to fumarate and sulfate. The hydrophobic APIs showed the lowest adsorption behavior to water vapor (<0.3%). These APIs were much more inert against water vapor. The reason is that the polar solvent only has limited access to the hydrophobic surface and pores [12]. However, it is assumed that the hygroscopic APIs (e.g. ITB) are difficult to examine using DVS-method development because the amorphous amounts might be very unstable instead of a high water uptake (even at low humidity).

A significantly higher absorption can be detected for the fully amorphous samples (Fig. 2B) even though the same measurement parameters were used (2 cycles, 0–90% RH, water). Due to the change in crystal lattice during the re-crystallization the second cycle shows a different curve progression for ITB, FF and SBS. The fully amorphous ITB sample showed an early re-crystallization (0.4 p/p_0, >4% change in mass) due to its hygroscopic characteristics. It is presumed that water in amorphous material is absorbed which therefore results in a higher water uptake [20]. This process enhances molecular mobility and facilitates the re-crystallization process [21,22]. For SBS and FF re-crystallization events were determined at 0.8 p/p_0 in combination with a very high water uptake (>10% change in mass). Bromide, fumarate and sulfate

Table 3
Physico-chemical characteristics of the investigated APIs.

	ITB	FF	SBS	CS	BS	GC
Molecular weight (g/mol)	430.4	478.5	576.7	540.7	430.5	494.0
Solubility	Soluble in H_2O	Slightly soluble in H_2O	Soluble in H_2O	Practically insoluble in H_2O	Practically insoluble in H_2O	Practically insoluble in H_2O
Melting point (°C)	237.9 ± 0.4	143.7 ± 1.1	210.7 ± 0.4/282.8 ± 0.5	210.4 ± 0.2	260.4 ± 2.1	173.1 ± 1.5
Re-crystallization (°C)	77.9 ± 4.2	105.9 ± 1.9	No event	135.2 ± 0.0	114.3 ± 1.5	102.5 ± 0.3
Glass transition (°C)	29.5 ± 1.3	46.4 ± 2.1	116.5 ± 1.6	96.1 ± 1.0	77.2 ± 1.1	61.0 ± 2.5
Density (g/cm³)	1.4091 ± 0.0042	1.3412 ± 0.0035	1.3424 ± 0.0044	1.1935 ± 0.0048	1.2890 ± 0.0049	1.3691 ± 0.0031
Surface energy (mJ/m²)	40.0 ± 0.5	51.2 ± 1.6	41.1 ± 2.0	41.0 ± 1.1	48.4 ± 0.8	41.7 ± 1.0
Water content (%)	3.65	2.28	0.47	0.46	0.38	0.29
Lipophilicity (Log P) Source: Drug Bank	−1.80	1.06	0.34	5.32	2.73	3.78
Drug class	Anticholinergic	β₂-Sympathomimetic	β₂-Sympathomimetic	Glucocorticoid	Glucocorticoid	Antidiabetic
Market product	Atrovent N®	Foradil®	Ventolin®	Alvesco®	Pulmicort®	Euglucon N®
Dose (µg)	20	12	100	80–160	200–400	3500
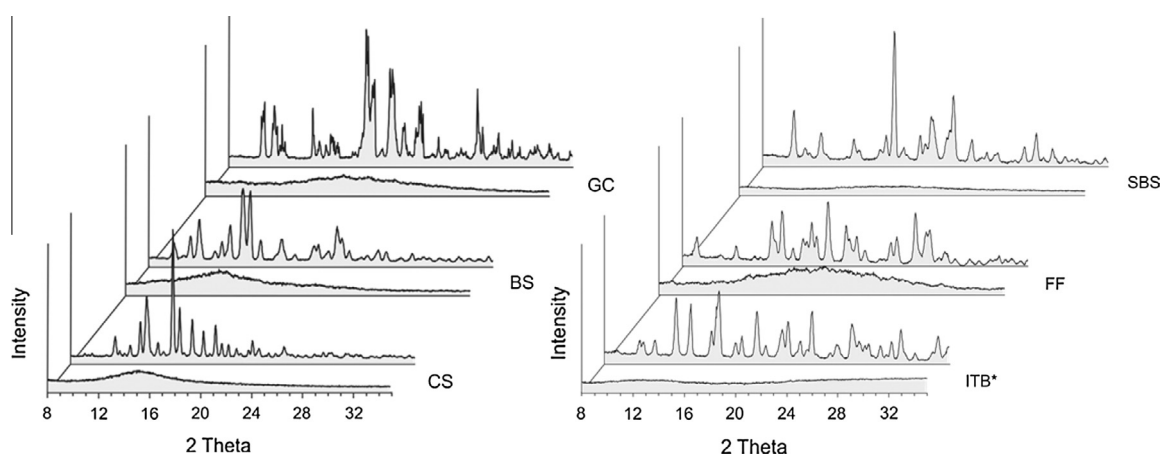
Fig. 1. XRPD patterns of crystalline and fully amorphous hydrophilic and hydrophobic APIs.

confirmed their behavior toward absorption – a higher water activity led to an earlier re-crystallization. Again the hydrophobic APIs underpin a lower affinity to water vapor, which can be found when fully amorphous. Moisture sorption isotherms of CS and GC resulted in the lowest values (<2% change in mass). BS showed a higher affinity to water vapor (<4.5% change in mass) which may also explain the low stability of amorphous amounts. For all hydrophobic APIs re-crystallization events were not detectable with water vapor, neither sudden loss in mass or a different curve progression for the second cycle was detected.

The results of moisture sorption isotherms can provide an indication which can be used for further measurement parameters (humidity levels) to have a balanced equilibration of mass. In particular the re-crystallization events for hydrophilic APIs (ITB, FF and SBS) are helpful in the development of the one-step DVS-HT method. After the drying phase of the sample the amorphous amounts have to stay stable without a re-crystallization during the measurement. For the hydrophilic APIs, all further screening measurements (DVS-HT) were taken with water as the solvent. Humidity levels were chosen below the determined re-crystallization events (Table 1). For SBS and FF p/p_0 values between 0.3 p/p_0 and 0.4 p/p_0 were tested (re-crystallization 0.8 p/p_0) and for ITB p/p_0 values between 0.1 p/p_0 and 0.2 p/p_0 were tested (re-crystallization 0.4 p/p_0). Test series were performed with micronized powders (JM1, Gc 1 to Gc 3) of each API, and a number of higher grinding cycles led to higher amorphous amounts and therefore to higher absorption values.

In particular the drying time had to be adapted and was increased for these hygroscopic APIs (FF: 30 h and ITB: 36 h). The increase of 10 or 16 h enables a balanced drying phase but long drying phases (>35 h) seem to be impracticable for a quick in-process monitoring because of a long DVS-measuring time (ITB > 2 days). For SBS and FF a high p/p_0 value of 0.4 was chosen on the basis of the re-crystallization point at a higher humidity. A total gas flow of 400 sccm (standard cubic centimeter per minute) and a temperature of 25 °C were chosen for all measurements. Fig. 3A gives an overview of the optimized parameters for the successfully investigated hydrophilic APIs (SBS and FF) and the corresponding sorption behavior. At the time of moisture increase, a sudden increase in mass (Y-axis 1) is visible and over the entire period a balanced mass was measured. This indicates a stable and calculable amorphous content for all three samples. Y-axis 2 shows the used p/p_0 value of the method for all three hydrophilic drugs.

Due to low water absorption (Fig. 2B) in hydrophobic substances a change from a polar to a nonpolar solvent was necessary. For CS powder samples isopropanol showed the best characteristics where a high mass absorption without an early re-crystallization was found compared to methanol, ethanol and ethyl acetate [10]. Different organic solvents were tested for GC and BS in order to receive a higher selectivity and higher slope of the calibration curve. Screening measurements were taken with water, methanol, isopropanol and methylene chloride as solvents (Table 1). As a prerequisite a balanced mass absorption is again
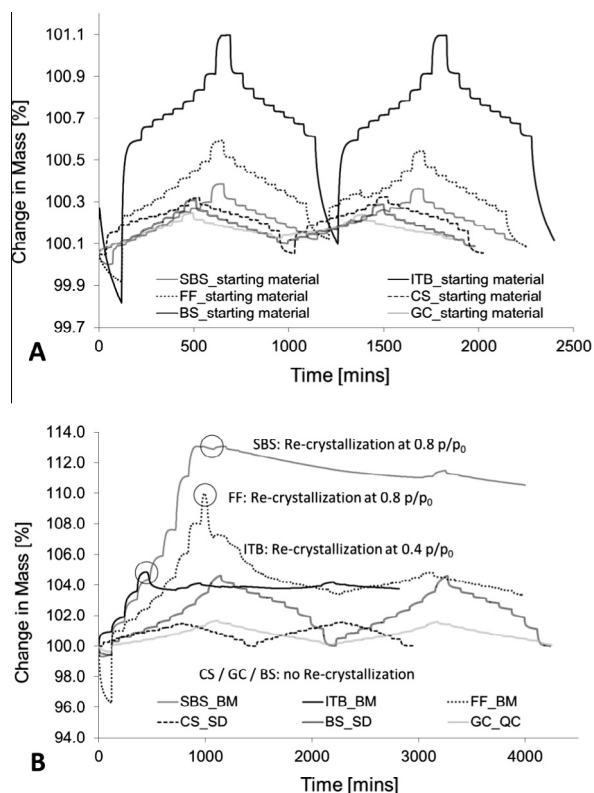
Fig. 2. DVS-1 measurements for crystalline (A) and fully amorphous (B) hydrophilic and hydrophobic APIs.
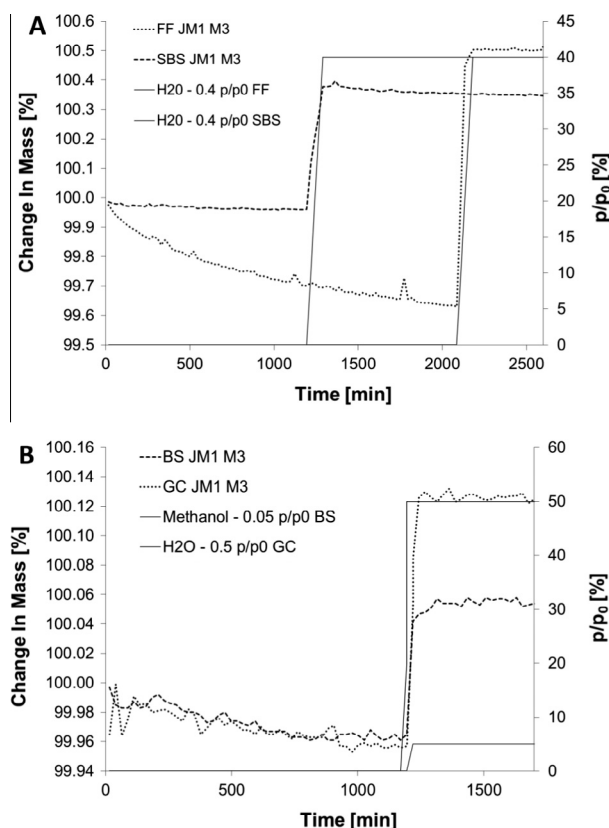

Fig. 3. Optimized DVS-HT measurements for the calculation of amorphous amounts for hydrophilic SBS and FF (A) and hydrophobic GC and BS (B).

necessary and different grinding cycles of each API may lead to different mass absorption.

Even at low humidity levels (0.05 p/p_0) very nonpolar solvents (e.g. isopropanol and methylene chloride) led to a fast re-crystallization for amorphous BS (Fig. 4A). The absorption was comparable to the crystalline starting material and indicates an unstable amorphous BS sample. Methanol was determined as the most appropriate solvent with an increased, stable mass absorption and the highest affinity to this hydrophobic powder (methanol > isopropanol > methylene chloride). Optimized vapor for methanol was a p/p_0 with the value of 0.05, and higher p/p_0 values of 0.2–0.3 can initialize a re-crystallization. For GC all solvents led to a balanced mass increase at 0.05 p/p_0, however, significant differences were not detectable in mass absorption (maximal 0.03%) due to the low affinity to all solvents (Fig. 4B). The absorption behavior was hardly increased in relation to the more nonpolar solvent (methanol < isopropanol < methylene chloride). At higher p/p_0 values of 0.1–0.2 a balanced mass adsorption could not be measured for methanol, isopropanol and methylene chloride (quick mass lost for all three solvents, Fig. 4B). It can be concluded that the amorphous amounts were not stable at these humidity levels.

Finally, isopropanol and methylene chloride showed a lower mass adsorption (0.05 p/p_0) compared to ddH$_2$O (0.5 p/p_0) due to its poor solubility (data not shown). The polar solvent demonstrated a significant difference in the absorption behavior when increasing the humidity level (10 times higher). As mentioned in the literature benzyl alcohol and dimethyl sulfoxide should increase the solubility, but cannot be used because of their toxicity in terms of personal safety, as well as device compatibility. It is recommended to measure at higher levels of water vapor (0.5 p/p_0) since none of the solvents show a high affinity to the hydrophobic GC. With this inconsideration raising the humidity level led to an extreme increase in mass and allowed a more precise determination of the stable amorphous content.
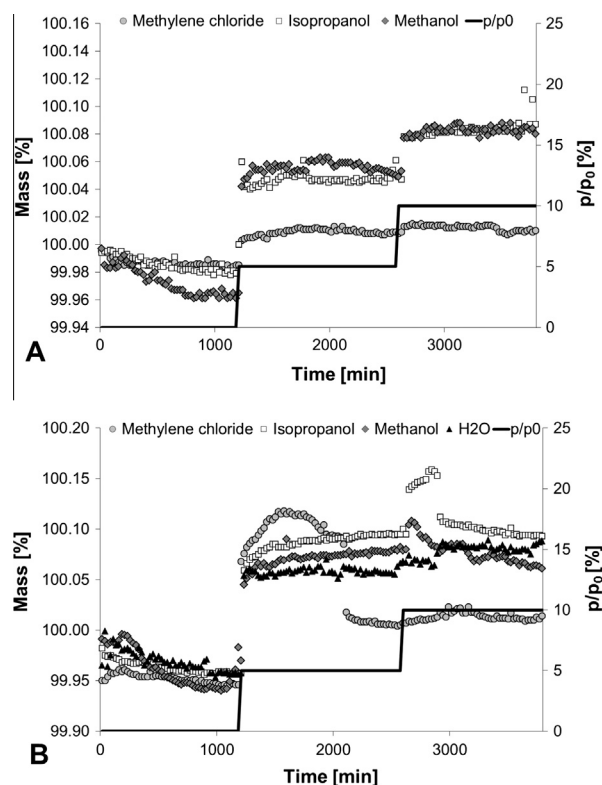

Fig. 4. Organic solvent screening for jet-milled BS (A) and GC (B) samples.

Fig. 3B shows the one-step measurement and corresponding sorption behavior of the hydrophobic samples (BS and GC). The amorphous amounts of the GC samples were more stable in comparison to the BS samples during the screening process. A total gas flow of 200 sccm and a temperature of 25 °C were used for all organic solvents. Table 4 summarizes the final parameters which were used for the determination of the amorphous contents, respectively.

After the measurement of the respective mixtures (0.25–15.0% amorphous content) it was possible to calculate the amorphous amounts in the unknown samples (jet-milled powders). The coefficient of determination (Fig. 5) shows that there is a very good linearity between mass absorption and mixed amorphous content for all APIs ($R^2 > 0.99$).

The APIs (FF and BS) that were available as micronized starting materials (Table 5), tend to electrostatic charge, and showed the lowest coefficient of determination ($R^2 = 0.9938$ and $R^2 = 0.9909$) and slight variations. It is assumed that different adhesion to the inner walls of a mixer leads to a lower homogeneity during the mixing process [23]. Furthermore, the effect of particle shape/size on powder flow and cohesion is well documented in the literature [24]. This presumption can be confirmed by a higher surface energy. Micronized FF showed a surface energy of 51.20 ± 1.58 mJ/m^2 and BS of 48.40 ± 0.77 mJ/m^2 compared to the crystalline starting materials with a surface energy of 40.04–41.70 mJ/m^2 (Table 3). Shah et al. postulated that powder cohesion increases in a linear correlation with an increasing surface energy of the API [25].

All samples were determined in duplicate in one measurement (10 samples per measurement). Only the hygroscopic ITB showed a low stability of its amorphous parts (data not shown), which was not by the measuring method. The mixture of the amorphous freeze-dried component absorbed moisture coming from the crystalline sample (>3.7% water content). This led to the re-crystallization process and resulted in a decreasing mass absorption. In this case a calibration is not possible and practicable.

Furthermore the process standard deviation (S_{x0}) and the process coefficient of variation (V_{x0}) of these calibration curves were calculated (Table 6). Hereby, the calculation of the spread of the measured values (n = sample size) of the calibration curve (SE, Eq. (1)) and the slope of curve (b), which represents the precision and the sensitivity, were used for this further calculation (Eq. (2)). The second parameter describes the relative measure of the precision including the process standard deviation (S_{x0}) and the mean of the concentrations (x-axis) (Eq. (3)). For these DVS methods a respectable precision was reached, respectively. Nearly all APIs showed a low process coefficient of variation for this new introduced method. Nevertheless FF and BS demonstrated an increased value.

$$SE = \sqrt{\frac{1}{n-2}\left[\sum (y-\bar{y})^2 - \frac{[\sum (x-\bar{x})(y-\bar{y})]^2}{\sum (x-\bar{x})^2}\right]} \qquad (1)$$

$$S_{x0} = \frac{SE}{b} \qquad (2)$$

$$V_{x0} = \frac{S_{x0}}{\bar{x}} \cdot 100\% \qquad (3)$$

Table 4
Final parameters for all used APIs for the determination of amorphous content by DVS.

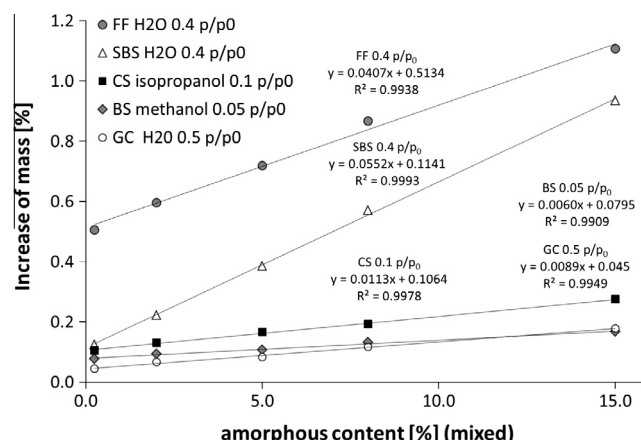| Samples	Solvent	Drying time (min)	p/p_0 value	Temperature (°C)	Total gas flow (sccm)
FF	ddH$_2$O	2160	0.4	25	400
SBS	ddH$_2$O	1200	0.4	25	400
BS	methanol	1200	0.05	25	200
GC	ddH$_2$O	1200	0.5	25	400

Fig. 5. Calibration curves of all investigated APIs for the calculation of amorphous amounts.

Table 5
PSD for the crystalline starting materials.

API	x_{10}	x_{50}	x_{90}	Span
ITB_Starting material	0.70 ± 0.02	1.81 ± 0.01	4.21 ± 0.04	1.95 ± 0.03
FF_Starting material	0.68 ± 0.01	1.58 ± 0.02	3.34 ± 0.05	1.69 ± 0.02
SBS_Starting material	2.03 ± 0.01	9.11 ± 0.07	33.33 ± 1.19	3.44 ± 0.11
CS_Starting material	4.74 ± 0.05	57.90 ± 2.96	288.91 ± 19.18	4.91 ± 0.16
BS_Starting material	0.59 ± 0.00	1.66 ± 0.02	4.57 ± 0.10	2.40 ± 0.04
GC_Starting material	7.31 ± 0.43	34.45 ± 0.39	85.38 ± 0.66	2.27 ± 0.02

Table 6
Process standard deviation of the calibration curves of the developed DVS methods.

Samples	SE (%)	S_{x0} (%)	V_{x0} (%)
FF	0.0216	0.5297	8.7557
SBS	0.0099	0.1799	2.9741
BS	0.0039	0.6459	10.6762
GC	0.0042	0.4766	7.8774

Finally, the jet-milled powders (8 bar, 1 grinding cycle up to 3 grinding cycles) showed for FF almost no amorphous amounts (<0.25%), however, amorphous amounts were measured from 1.5%, 4.2% and 5.1% for SBS. The amorphous amounts for hydrophobic APIs resulted in 5.5%, 14.0% and 17.0% for CS, almost no amorphous content (<0.25%) for BS and finally 5.7%, 7.7% and 8.5% for GC.

3.4. Optical investigation of the particle morphology – SEM pictures

Optical investigations of crystalline and micronized APIs (BS/GC) were conducted to find significant differences in particle morphologies of these hydrophobic powders (Fig. 6). These pictures may clarify important interactions with the organic solvents and explain mechanisms during the measurement of the one-step DVS method. The storage was carried out in a desiccator (2.4 l, room temperature) for 2 h over the organic solvent, respectively. Hereby, no significant change in PSD was determined. In this part of the study the respective solvents from the organic solvent screening of the DVS measurements were used again.

It is obvious that BS_JM1_GC3 indicates a very smooth surface after solvent storage with methanol. Particle corners are rounded and touching particles are melted together. This behavior might prove to be advantageous for higher absorption values and affect a precise detection of the amorphous content. This finding was similar to the CS samples (storage over isopropanol) and their

determined smooth surface after the solvent storage [6,10]. A significant change in particle morphology was not observed for GC samples during conditioning due to poor affinity to solvents such as isopropanol and methylene chloride.

4. Discussion – general instructions for method development: Determination of amorphous amounts by DVS

Before starting with the method development it is necessary to investigate basic material properties of the APIs. Solubility, thermal events and surface properties play a major role. In a second step completely amorphous samples are to be prepared for calibration. As a medium of choice a ball-mill was used to convert the crystal lattice. Hereby, an optimized grinding time, rotation speed, number of grinding balls and the filling level have to be carefully determined. As other methods, spray drying, freeze-drying or quench cooling can be used for the production of fully amorphous samples. XRPD and DSC analytics determine the absence of crystalline material in the amorphous samples. In a third step jet-milled samples with different grinding cycles are to be prepared for further
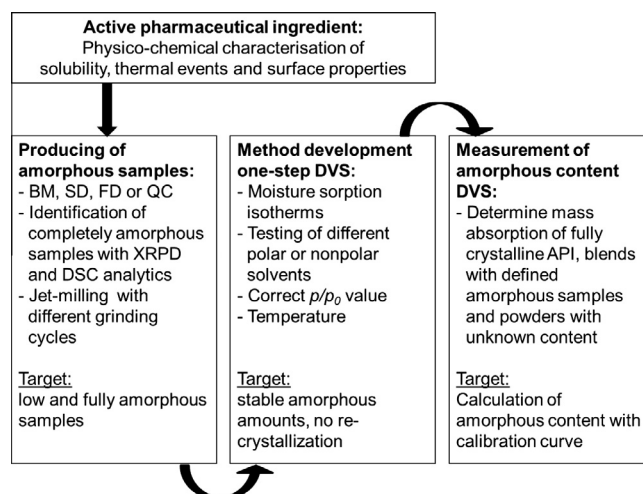
Fig. 7. Overview of method development: Production, test measurement and calculation.
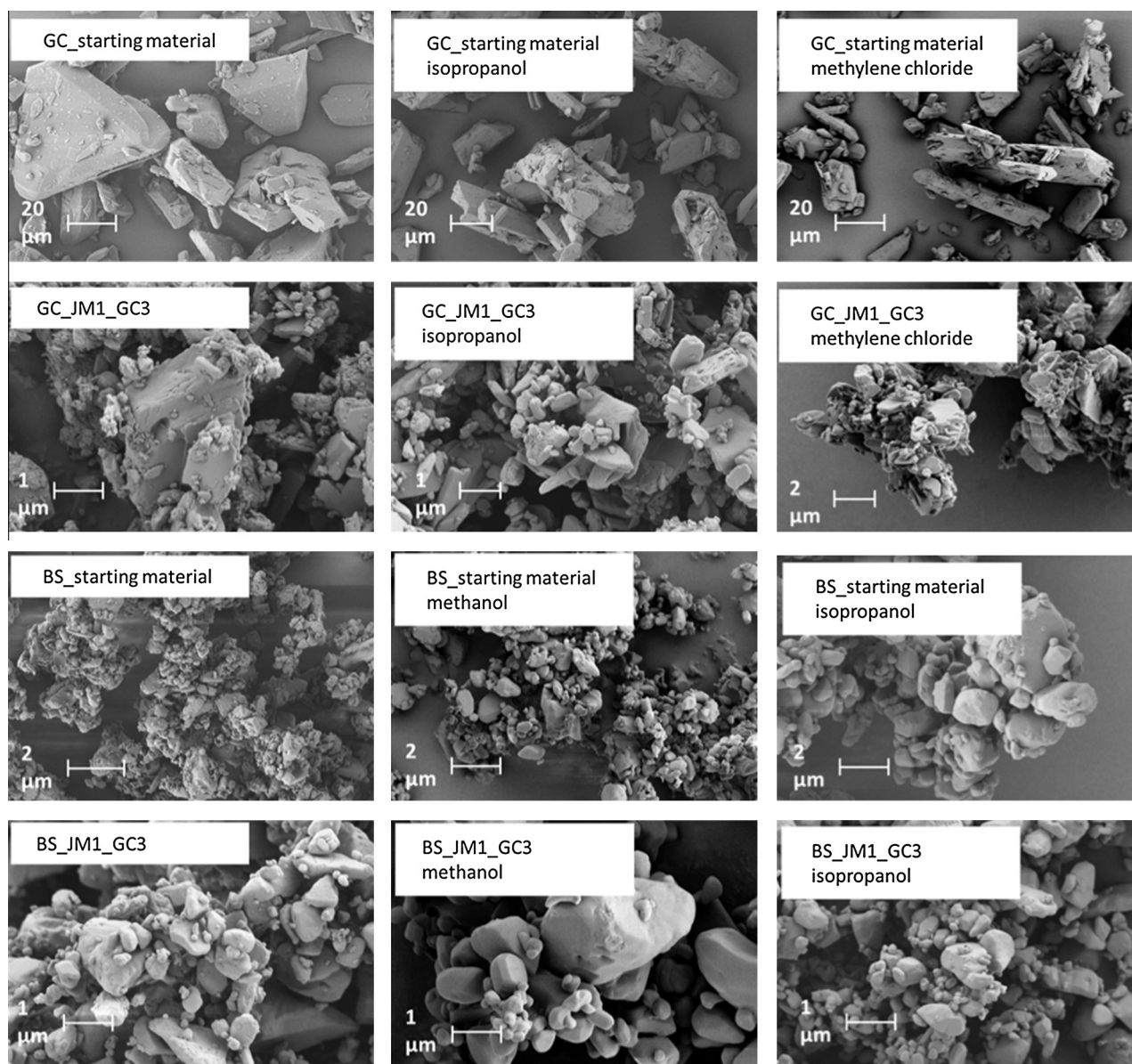
Fig. 6. SEM pictures of hydrophobic APIs (BS/GC) stored in desiccators with different solvents.

screening DVS-measurements (low amorphous amounts). Different polar or nonpolar solvents are to be tested and finally different p/p_0 values are measured to find a balanced mass absorption without re-crystallization. Of course, the influence of the temperature cannot be ignored due to physico-chemical characteristics of the solvents (e.g. measuring at 20 °C for methylene chloride because of low boiling point at 40 °C). Ultimately with the determined conditions the crystalline starting material and the blends of fully amorphous and crystalline material (calibration curve) are measured. Due to the calibration, in further course unknown samples can be measured and the amorphous amount can be calculated. Fig. 7 shows an overview of a general guidance.

5. Conclusion

This DVS study demonstrates with a high accuracy how amorphous regions are specifically detectable as "reactive spots" [26] to vapor. The amorphous content is the critical outstanding factor and becomes the deciding part of nearly the total signal [27] distinguished to various analytical techniques (e.g. DSC and XRPD measurements). It was shown that the hydrophilic and hydrophobic APIs behave differently, in this case the affinity of the drug to the solvent plays an important role. Once hydrophilic APIs show high initial water content, these APIs (ITB and FF) might interact well with vapor (high adsorption values: >0.5% for crystalline material). This behavior leads to a fast lowering of the glass transition temperature and an associated re-crystallization for induced amorphous amounts. In practice a re-crystallization for hydrophilic, micronized powders is easily realized only by water vapor. In comparison SBS showed measureable and stable amorphous amounts (up to 5.1%).

The hydrophobic APIs demonstrated as well significant differences for the adapting of the one-step DVS method and the stability of amorphous amounts. In this study BS had to be measured with an organic solvent (in this case: methanol) and at low humidities (0.05 p/p_0). In contrast GC showed a low affinity to all used organic solvents and the stable amorphous amounts were detected with an increased water vapor (0.5 p/p_0). In summary, the determined amorphous amounts for GC (5.7% up to 8.5%) are long-term stable and may not easily switch to their crystalline counterparts compared to the BS samples.

In general, it is assumed that amorphous parts can influence drug delivery (dissolution behavior) and product stability (shelf life) because of the induced particle size changes (increase of particles) by uncontrolled re-crystallization processes. In particular amorphous parts in inhalation products have to be carefully examined and prevented in large extent for an efficient pulmonary drug delivery in the lower respiratory tract.

In summary, it is always necessary that these amorphous parts are precisely determined and re-crystallized after the production process under controlled conditions with suitable solvents. This approach may facilitate the specifications for in-process controls in the pharmaceutical industry.

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