



Amorphous Content Determination using Amorphous and Crystalline Standards

S. Molisso, M. Naderi, M. Acharya, M. Guo, C. Hewson and D. Burnett

Surface Measurement Systems Ltd.

This application note will discuss methods to measure amorphous contents of pharmaceutical materials, focusing on the DVS method devised by Mackin et al. Amorphous contents can be determined using this method via measurement of solvent uptake before and after a crystallization event with a limit of detection as low as 0.5% w/w.

Introduction

Amorphous materials present complex and challenging difficulties for pharmaceuticals due to their unpredictability associated with their processing, storage, and ultimate performance of the product. While some amorphous content may be desirable to improve the solubility of a drug, it can also be generated unknowingly during processing via milling, wet granulation or spray drying. It has been well established that less than 10% amorphous content can drastically alter the properties of a powder, which necessitates the use of sensitive techniques to measure these small amorphous quantities.¹

There is an abundance of analytical tools capable of measuring the amorphous content of both pharmaceutical products. The following section will describe and discuss the advantages and limitations of some of these methods.

In thermal techniques, Isothermal Gas Perfusion Calorimetry (IGPC) uses the heat of crystallization to quantify the amorphous content of pharmaceutical powders. Like many other techniques to be discussed, this requires a calibration curve of known amorphous standards, which in IGPC can often be found to be non-linear at higher amorphous contents.² Alternatively, the

heat of adsorption can be directly correlated to amorphous content and may be a more reliable method due to the non-dependence on crystallization kinetics.² Both methods show low limit of quantification at 5.28% w/w and 0.92% w/w respectively.²

Differential Scanning Calorimetry (DSC) is often used for amorphous quantification. However due to the small energy changes associated with the measurement of glass transition at low levels of amorphous contents, it is not suitable for measurements of amorphous contents below 10% w/w.³ Hyper-DSC uses faster scan rates than normal DSC, increasing its sensitivity to glass transition events and resulting in measurements with a limit of quantification of 1.89% amorphous content.³ Polymorphs make DSC analysis difficult due to their differing melting enthalpies which may lead to misleading results in amorphous content determination.

Dielectric Thermal Analysis (DTA) can be used to measure the electrical ionic conductivity of an amorphous active pharmaceutical ingredient (API) within a crystalline solid, due to the low conductivity of crystalline solid and high conductivity of amorphous fractions.⁴ Experimental results however are limited to





detection of high amorphous contents (>50% w/w) and there is no data available on low amorphous content solids.⁴

Solution Calorimetry is another frequently used thermal technique, measuring the enthalpy of dissolution which can be correlated to the amorphous content.⁵ While multiple factors lead to dissolution and can make the interpretation of results complicated,⁶ excellent linearity can be observed between the enthalpy of dissolution and amorphous content. This results in limits of quantification as low as 3.5% and 3% for physical mixtures and sprayed dried samples of lactose respectively.⁵

Other spectroscopies include Fourier Transform-Raman, which while has advantages in directly probing chemical structure, is limited by the small surface area measured using the laser.⁶ Raman methods have been shown to have a limit of detection of 3.48% w/w in pharmaceutical materials.⁷ Spectroscopic methods such as Raman and Solid-state NMR (limit of quantification for ss-NMR is approximately 0.5% w/w) are superior to thermal techniques in their non-destructive nature, however the broad amorphous peaks generated makes these spectroscopies better suited to the measurement of crystalline content.⁶

Powder Xray Diffraction (PXRD) can measure amorphous contents as low as 5% w/w using the integration of diffraction peaks.^{6,8} This is one of the most widely used techniques to measure amorphous contents of solids, however data analysis may be subjective due to the positioning of the baseline in noisy diffraction spectra. Similar to DSC, PXRD has limitations in terms of polymorphs, as polymorphs will differ in diffraction patterns. Finally, PXRD typically needs a large amount of sample, however this can be recovered.

There is still a need for methods that can measure amorphous contents below 0.5% w/w. Some of the most sensitive techniques used to accomplish this include dynamic vapor sorption

(DVS), which has been demonstrated to have limits of detection better than 0.5% w/w.⁹

Quantification of amorphous content with DVS relies on the principle that amorphous solids undergo bulk absorption as well as surface adsorption, whereas crystalline materials only undergo surface adsorption.

Numerous methods have been described using DVS to measure amorphous content and can be found in detail elsewhere¹⁰⁻¹⁵. In this application note however, the focus is on the method described by Mackin *et al.*¹⁶, where crystallization of the amorphous phase is induced, and the solvent uptake before and after the crystallization event measured. The difference between sorption before and after the crystallization event, is directly related to solvent absorption into the amorphous bulk, and therefore directly related to the amorphous content of the sample.¹⁶

Theory

Due to this methods reliance on a crystallization event, a suitable solvent must first be chosen which can induce crystallization. For Fluticasone Propionate (Figure 1), this solvent is ethanol. Figure 1 demonstrates that as the compound is exposed to 90% P/Po ethanol there is a mass drop, associated with a crystallization event.

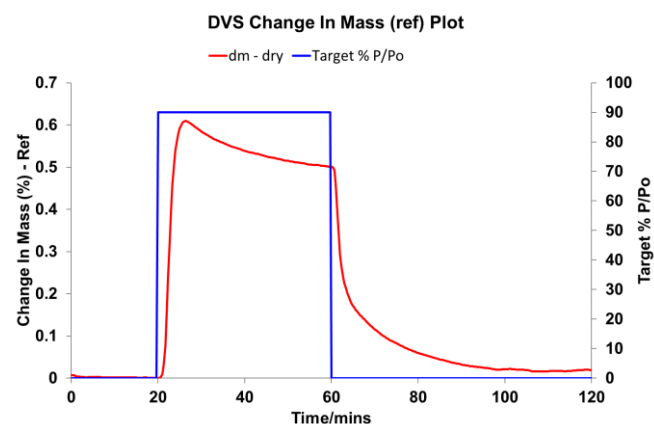


Figure 1 The crystallization of Fluticasone Propionate in ethanol.

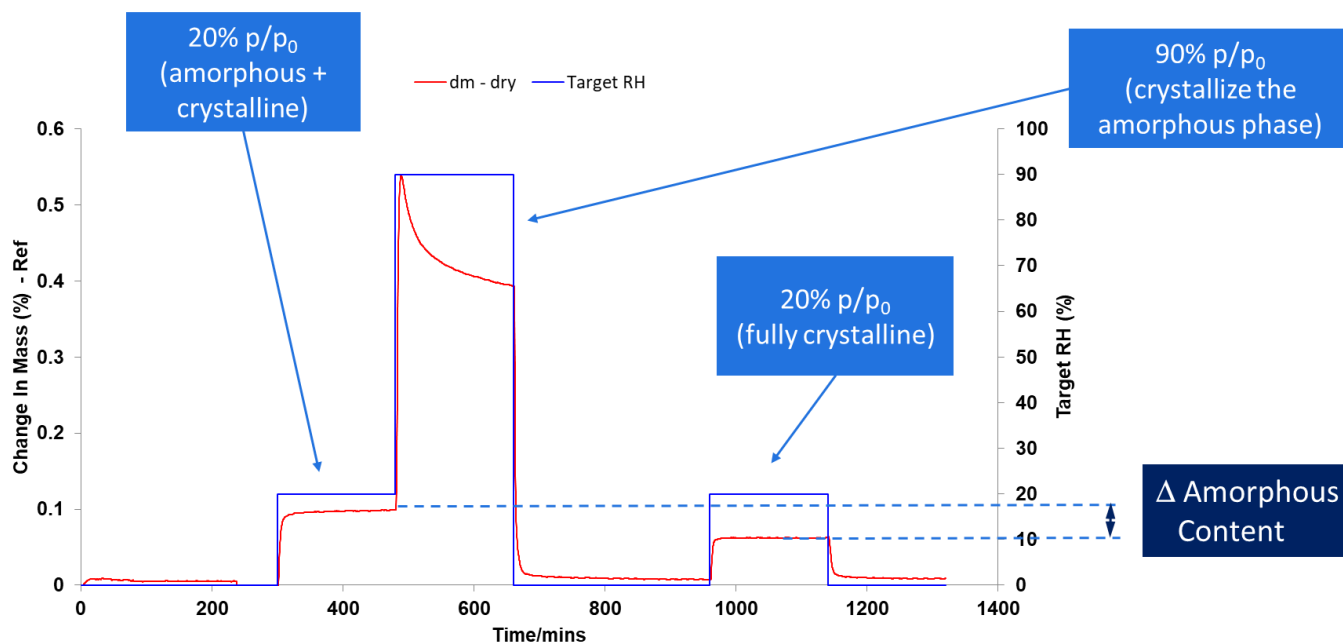


Figure 2 The DVS experiment for amorphous content determination.

The amorphous content determination experiment has 6 stages:

1. An initial drying stage
2. A stage exposing the sample (both the amorphous and crystalline phases) to a partial pressure of vapor known *not* to induce crystallization
3. A stage at a partial pressure of the vapor that *does* cause crystallization of the amorphous phase
4. A drying stage
5. A second exposure of the partial pressure at step 2.

In step 2, sorption is accounted for by surface sorption onto the crystalline phase, and both surface and bulk sorption into the amorphous phase. In step 5 however, when the amorphous phase has been crystallized, the total vapor sorption is only resultant of surface adsorption. Hence, the difference in sorption capacity between step 2 and step 5 directly correlates with the amorphous content in the sample.

Once this experiment has been performed for several standards of known amorphous content, the DVS Analysis Suite can be used to calculate

the amorphous content of a sample with an unknown amorphous content. This method is one of four available methods to determine amorphous content in the DVS Analysis Suite (Figure 3).

Method

Fluticasone Propionate (Figure 4) is a corticosteroid used to reduce inflammation and treat asthma.

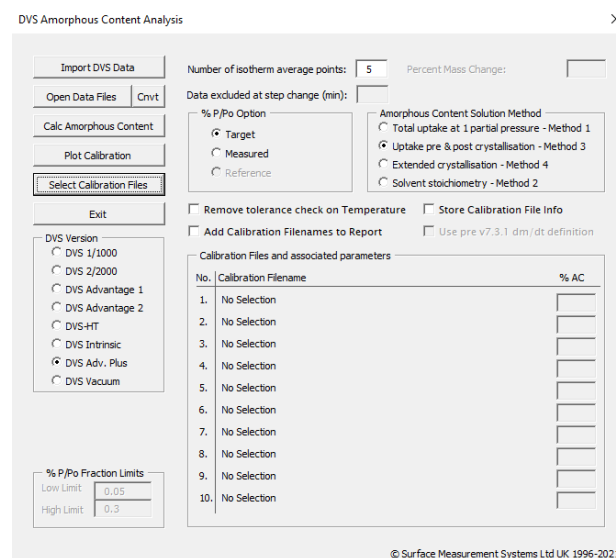


Figure 3 The DVS Amorphous Content Analysis Suite

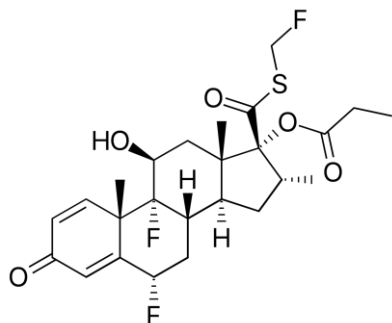


Figure 4 Chemical structure of fluticasone propionate

In this study a sample of fluticasone propionate with an unknown amorphous content was investigated. To determine the amorphous content, the standards used included a 2% amorphous, 5% amorphous, 10% amorphous and fully crystalline standard of fluticasone propionate. Sample sizes ranged between 50-100mg, and all experiments were performed at 25°C with a flow rate of 100sccm. Ethanol was chosen as the solvent to precipitate crystallization. The DVS method is displayed in Table 1, with a description of each stage.

Results

Figure 5 shows the DVS results for the experiments, of unknown amorphous fluticasone propionate, a fully crystalline standard and a 2% amorphous standard. First the sample is exposed to 20% P/Po ethanol, then 90% P/Po which induces crystallization of the amorphous phase.

This is seen in step C with a large drop in mass characteristic of crystallization. In step E there is an appreciable difference in sorption to step B. This difference is larger than for the 2% amorphous content standard, making it clear that the unknown amorphous content of the fluticasone propionate material is higher than 2% w/w.

Table 1 DVS method for amorphous content determination

Stage	Stage Length	Partial Pressure Ethanol	Stage Description
A	0 hr – 5 hr	0% P/Po	Drying Stage
B	5 hr – 8 hr	20% P/Po	Exposure to amorphous and crystalline state
C	8 hr – 11 hr	90% P/Po	Crystallization of amorphous phase
D	11 hr – 16 hr	0% P/Po	Drying Stage
E	16 hr – 19 hr	20% P/Po	Exposure to fully crystalline state
F	20 hr – 22hr	0% P/Po	Drying Stage

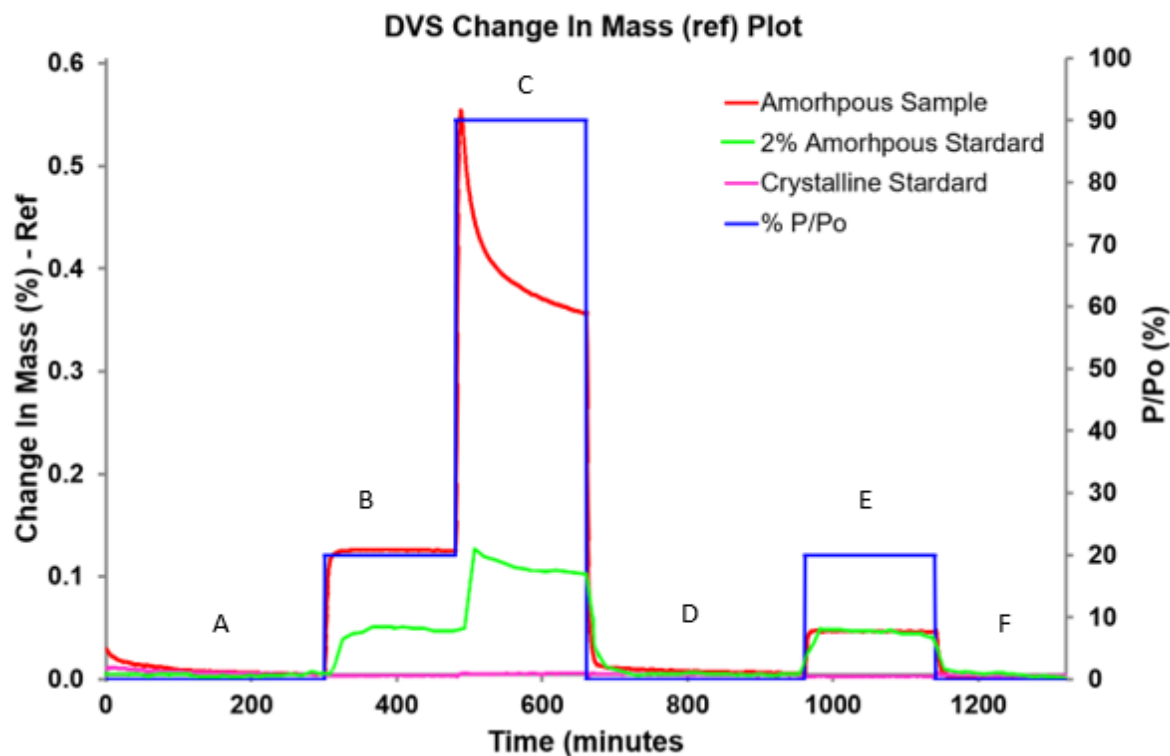


Figure 5 DVS data for determining the amorphous content of fluticasone propionate. Red is the amorphous sample mass, green a 2% amorphous standard, and pink a fully crystalline standard. Each stage label is found in Table 1.

The fully crystalline standard shows almost no change in uptake during the entire experiment, as would be expected for surface only adsorption.

Using the DVS Analysis Suite, a calibration curve using the fluticasone propionate standards was calculated (Figure 6), and the amorphous content for the unknown fluticasone propionate was calculated to be 6.3% w/w (Figure 7).

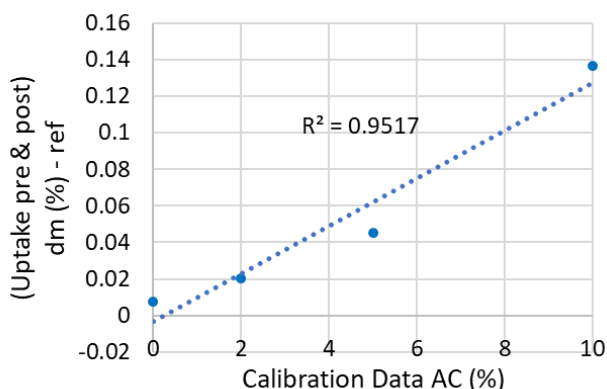


Figure 6 The calibration curve for amorphous content using fluticasone propionate standards

Calibration Data (Uptake pre & post)	
AC (%)	dm (%) - ref
0.0	0.0075
2.0	0.0206
5.0	0.0451
10.0	0.1364
Sample (calc at RH of 20.0)	
AC (%)	dm (%) - ref
6.3	0.0795

Figure 7 DVS Analysis Suite results showing the calculated amorphous content of the fluticasone propionate from the amorphous standards calibration data.

Conclusion

There are many different methods available to measure the amorphous content of pharmaceutical materials, but few have the ability to measure amorphous contents below 10% w/w. In this application note, a DVS method devised by Macklin *et al.* was used to determine the amorphous content of an unknown amorphous



sample of fluticasone propionate using known amorphous and crystalline standards. This sensitive and straightforward method can measure amorphous contents as low as 0.5% w/w.

Common Questions

Q) What vapour should be used for the experiment?

A) A vapour should be chosen that induces crystallization of the sample, this can be water, or organic vapours as long as it does not cause deliquescence of the material.

Q) How long should each DVS stage be?

A) As this depends on how quickly the sample crystallizes, it is recommended that a dm/dt of 0.002% is used to determine full equilibrium after each stage.

Q) Can this DVS method be used to quantify large amorphous contents?

A) Yes, however this may lead to longer experimental times due to the extended periods needed for crystallization of a larger amorphous phase.

Q) Why does the amorphous phase take up more solvent?

A) Amorphous regions can absorb solvent into the bulk of the amorphous phase, as well as adsorb solvent to the surface. Crystalline materials can only adsorb solvent vapours, leading to a reduced total uptake in comparison.

Q) Is an internal standard needed for this measurement?

A) No, only a calibration curve is needed, made from samples of a known amorphous content.

Q) How much sample is needed?

A) If the sorption capacity for the sample is less than 0.5% w/w, approximately 50-100mg of sample is appropriate. If sorption is greater than 10% w/w, then a smaller sample size of 10-20mg can be used.⁹

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Head Office:

Surface Measurement Systems, Ltd
5 Wharfside, Rosemont Road
London HA0 4PE, UK
Tel: +44 (0)20 8795 9400
Fax: +44 (0)20 8795 9401
Email: science@surfacemeasurementsystems.com

United States Office:

Surface Measurement Systems, Ltd, NA
2125 28th Street SW, Suite I
Allentown PA, 18103, USA
Tel: +1 610 798 8299
Fax: +1 610 798 0334

