The Application of Near Infrared Spectroscopy and Dynamic Vapor Sorption to Quantify Low Amorphous Contents of Crystalline Lactose

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Purpose. To explore the use of a combined near infrared spectroscopy and gravimetric sorption apparatus in providing an accurate quantification of amorphous contents of predominantly crystalline lactose.

Methods. Key wavelengths on the near infrared spectra of amorphous and crystalline lactose were used to construct a calibration plot of spectral fit to amorphous content. The extent of water sorption and desorption at 75% relative humidity (RH) was used to quantify the amount of amorphous material in the sample.

Results. Near infrared spectroscopy was used to quantify the amorphous contents of a set of 10 partially amorphous lactose samples using a calibration equation generated from an independent set of 17 samples. The results were found to be accurate to within 1% w/w amorphous content. Dynamic vapor sorption quantification relates the mass of water sorbed and subsequently desorbed during the crystallization process with the amount of amorphous material originally contained within the sample. It was possible to quantitatively detect as little as 1 mg of amorphous content in the sample. The percent amorphous content determination will thus be sample mass dependent, however, assuming a sample mass of 150 mg, the best detection would be ca. 0.7%.

Conclusions. It has been found that both techniques may be used to quantify small quantities of amorphous material. The combination of the two techniques lends itself to added verification of results and thus increased reliability.

KEY WORDS: amorphous lactose; quantification; near infrared spectroscopy; dynamic vapor sorption.

INTRODUCTION

It is known that some pharmaceutical processing techniques, e.g., milling (1), can induce changes in the crystallinity of materials, rendering them partially amorphous. It is also widely accepted that amorphous material induced in this way, although perhaps occupying only a very small proportion of the bulk, may contribute greatly to the behavior of that material as it resides primarily on the particle surface. This phenomenon has a great impact most notably on inhalation products, where micronization can dramatically alter surface characteristics. In addition, amorphous material is thermodynamically unstable and therefore liable, under certain circumstances such as elevated temperature or relative humidity (RH), to undergo changes in its amorphous structure, or to revert to the stable crystalline form.

Quantification of amorphous contents of materials is possible with techniques such as differential scanning calorimetry (DSC) and X-ray powder diffraction (XRPD). However, their lower limit of detection is at approximately 10% w/w amorphous content (2). Isothermal microcalorimetry (IMC) has been proved capable of detecting amorphous material at 0.313% w/w (3), however, when the desired outcome is an accurate quantification of that amorphous content, problems arise with this technique. With IMC, the sample is made to crystallize by exposure to a suitable environment (often high RH) and the heat change is measured (4). Quantification can be attempted by using the net heat change as a direct indicator of crystallinity. Samples with small amorphous contents crystallize with a much shorter lag time than samples of greater disorder, because less water sorption is required to induce crystallization (3). Often in these cases there is insufficient time to establish a steady baseline before the crystallization event, which can lead to subsequent problems with peak area determination. To delay the crystallization event, a greater sample size of perhaps several hundred milligrams is required, which can be a limitation in certain cases, for example when dealing with a new drug substance. Even assuming that a complete peak is produced, the quantification of that crystallization response through peak area measurement is problematic (5). It is therefore necessary to find alternative methods that can be used to quantify the amorphous content of predominantly crystalline powders.

Near infrared spectroscopy (NIRS) is a noninvasive technique, which requires no sample preparation; it is also nondestructive, enabling complete sample retrieval. To date, uses of NIRS have included material identification in quality control and the monitoring of bulk during manufacture or processing in order to obtain on-line information, such as achieving a suitable drying end-point. Physical and chemical information may be obtained, such as polymorphism (6) and mutarotation (7). Buckton et al. (7) have shown that it is possible to monitor the crystallization of amorphous lactose in real time through examination of NIR spectra at certain wavelengths.

Dynamic vapor sorption (DVS) provides extremely accurate gravimetric data in conjunction with a control of RH and temperature. Changes in RH can be used to cause amorphous materials to crystallize, with a consequent expulsion of water. Water sorption is a useful method by which to study the amorphous form either as a single component or in combination (8,9). Despite the fact that the extent of water sorption (and desorption) relates to the amorphous content of the sample, there has been little progress in using DVS to quantify the amorphous content of samples since the first study of Buckton and Darcy (10).

Recently, NIRS and DVS have been combined, by production of an NIRS probe that can be housed immediately

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ABBREVIATIONS: RH, relative humidity; DSC, Differential scanning calorimetry; XRPD, X-ray powder diffraction; IMC, isothermal microcalorimetry; NIRS, near infrared spectroscopy; DVS; dynamic vapor sorption; SNV, standard normal variate; RSS, residual sum of squares; SEC, standard error of calibration; SEP; standard error of prediction; y_i, actual amorphous content; Y_i, NIRS calculated amorphous content; n, number of samples; p, number of coefficients in the calibration equation.

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below the sample pan of the DVS (10). This allows both the drying of samples to a suitable endpoint, enabling the elimination of water-related interference in NIRS, and the humidification of samples with the ability to monitor the consequent changes in physical form with the NIRS. Lane and Buckton (11) have shown that the DVS-NIRS hyphenated system operates to a high performance standard, as data generated using the hyphenated DVS-NIRS technique were similar to those generated when using each technique separately.

In this current study, NIRS has been used to assess the amorphous content of samples when dry (within the DVS-NIR apparatus), and the DVS data have been used to assess the amorphous content by calculation of the extent of water sorption and desorption. The aim of the study was to establish the detection and quantification limits of the two techniques when used to assess the amorphous content of the same samples.

MATERIALS AND METHODS

Preparation of Amorphous Lactose

α-Lactose monohydrate was supplied by Borculo Whey Products UK Ltd., Cheshire, U.K. Production of amorphous lactose was achieved by spray-drying a 10% w/v aqueous lactose solution in a Buchi 190 spray dryer, following the parameters set out by Chidavaenzi et al. (12). The yield was collected immediately and stored over phosphorous pentoxide (Avocado, U.K.) under vacuum at room temperature. Confirmation of the amorphous nature of the yield was obtained from XRPD (Philips PW3710 X-ray powder diffractometer, Cambridge, UK) (data not shown).

Preparation of Amorphous Lactose Mixes

Crystalline lactose was found to be free of amorphous content as it did not show any crystallization response when exposed to humidity in the DVS, consequently it was used without conditioning. Quantities of dried amorphous and crystalline lactose were sieved to produce a particle size of less than 425 μm and then accurately weighed and combined to produce powder mixes of 0–10% w/w amorphous lactose, increasing in increments of 1%. The samples were subsequently mixed in a Turbula mixer for 20 min to ensure a homogeneous amorphous content under ambient conditions of 293K (±2K) and 50% ± 5% RH. Mixed samples were then stored over phosphorous pentoxide under vacuum at room temperature to ensure that moisture vapor was absent and to prevent changes from occurring in the amorphous material.

NIRS/DVS

Gravimetric studies of the amorphous lactose mixes were carried out in a humidity- and temperature-controlled microbalance DVS apparatus (Surface Measurement Systems, U.K.). Samples of approximately 35–40 mg were dried at 0% RH at 298 K. Six hours was generally sufficient for complete drying to be achieved, although it was possible to monitor and adjust this drying time as required. NIR spectra were recorded on the dry samples (Foss NIRSystems, U.K.) using a fiber optic probe situated approximately 4 mm below the flat-bottomed quartz glass DVS sample pan. NIR absorbance spectra for all samples were recorded as log (1/R), where R is reflectance. The NIRS instrument recorded the mean spectrum of 32 scans (taking approximately 40 s), over the wavelength region 1100–2500 nm. NIRS data processing and analysis was carried out using the Vision® software (Version 2.21) (©Foss NIRSystems, U.K.).

After the lactose was dried and NIR spectra were recording, samples were exposed to an elevated RH of 75% at 298 K, in order to induce crystallization. The mass change during this water sorption phase was recorded using the DVS as rate of change of mass (dm/dt) as a function of time. The area under the curve was calculated and recorded as the mass of water sorbed. As crystallization progresses, water is rapidly expelled from the sample. This water desorption was also recorded as dm/dt as a function of time and the mass of water desorbed was determined.

RESULTS AND DISCUSSION

NIRS Quantification of Amorphous Lactose

The NIR absorbance spectrum for each dry sample was entered into the Vision database along with its actual amorphous content known from mixing. These data were treated mathematically by performing a standard normal variate (SNV) transformation to remove multiplicative interferences of scatter and particle size. The first derivative was then calculated, which enabled enhancement of peak resolution (segment size of 10 nm and a gap size of zero data points). The spectra of the 28 dried amorphous samples used in this study (taken at the end of the drying step at 0% RH in the DVS) were randomly assigned to two groups in a 60:40 ratio—the former group forming the calibration set and the latter the validation set. A forward search multiple linear regression (MLR) was applied to the data in the calibration set using the Vision® software. It is designed to scan all wavelengths and select the one at which the best correlation for the samples between their actual amorphous contents and those calculated by NIRS is achieved. In this case that wavelength was found to be 2024 nm. A multiple correlation coefficient (R^2) between the actual amorphous contents and the NIRS values was found to be 0.927 at this wavelength. When a second wavelength (1970 nm) was added to the calibration, this value improved to $R^2 = 0.973$. A third wavelength of 1994 nm was added to further improve the correlation to $R^2 = 0.991$. No further wavelength was added to the calibration equation due to the risk of overfitting the data. The residual sum of squares (RSS) of the three wavelength calibration fit was found to be 1.47 (eq. 1), resulting in a standard error of calibration (SEC) of 0.350% w/w (eq. 2).

$$\mathrm{RSS} = \sum_{i=1}^{n} (y_i - Y_i)^2 \tag{1}$$

$$\mathrm{SEC} = \sqrt{\frac{\mathrm{RSS}}{n-p}} \tag{2}$$

where y is the actual amorphous content (known from preparation), Y is the NIRS calculated amorphous content, n is the number of samples, and p is the number of coefficients in the calibration equation.

To validate the calibration equation, it was used to quantify the amorphous content of those samples in the validation

set (Fig. 1). The standard error of prediction (SEP) of this set was found to be 0.84 % w/w (equation as for SEC where $p = 0$).

An indication of the accuracy of this technique can be gained from the SEC and SEP values calculated (0.35% w/w and 0.84% w/w respectively), which indicate how closely the NIRS results correlate with the known amorphous contents of the samples from preparation. Obviously the lower these values are, the better the calibration model. It would be expected that the SEP value be greater than the SEC value, because the calibration equation is based only on those samples in the calibration set.

Given the discussion of error above, the percentage error of the calculated amorphous content will decrease with increasing amorphous content (the absolute error staying the same). However, the error is sufficiently small that this technique offers the capability of quantifying amorphous contents to a high degree of accuracy, even if the amorphous content drops to around 1%. Bearing in mind the problems associated with IMC quantification and the speed, ease of operation, and accuracy of NIRS, this spectroscopic technique should supersede calorimetry in the quantification of low amorphous contents.

Quantification of Amorphous Content from DVS Sorption/Desorption Data

Following quantification of the amorphous content by NIRS, it was possible to change the RH in the DVS-NIR and use the mass change to enable the quantification of amorphous content by another method. The use of two methods in series on the same sample (the first being nondestructive) provides an instant check for internal consistency, which is very appealing.

From the DVS data it was possible to calculate the rate of change in mass (dm/dt) as a function of time for each amorphous mixture, starting from the point of exposure to 75% RH. Figure 2 is a typical DVS plot and shows a swift moisture uptake, due to vapor sorption at the elevated RH, followed very quickly (approximately 3 min) by a mass loss while the sample was still maintained at the elevated RH. This mass loss is due to crystallization, which is a consequence of the sorbed water plasticizing the amorphous material such that its glass transition temperature is reduced to below that of the environment at which the experiment is being performed (10). The expulsion of water during crystallization is not complete, as some of the water is retained as a partial monohydrate (some of the material crystallizes as an anhydrous form).

The area of the dm/dt plot that is above zero (see Fig. 3) corresponds to the water sorbed before weight loss starts and the point where the dm/dt plot crosses the x-axis is equivalent to the top of the peak of the sorption response. Consequently, the area under the positive peak of the dm/dt plot is the quantity of water sorbed during the sorption region of the response, whereas the area under the negative region of the dm/dt plot is the quantity of water desorbed. A linear relationship was obtained between the mass of sorbed water and

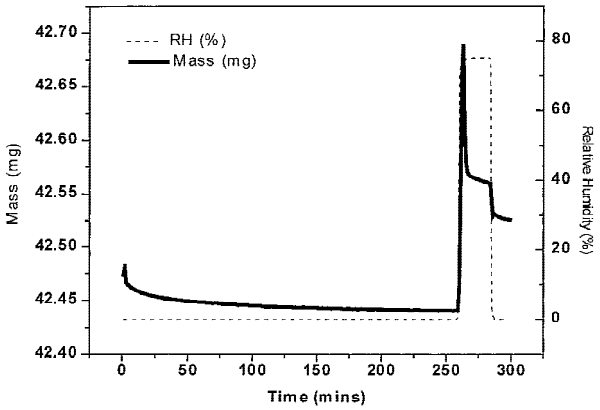

Fig. 2. Plot of mass against time for a 5% w/w amorphous lactose sample being dried at 0% RH for 260 min, followed by exposure to 75% RH in order to induce crystallization.

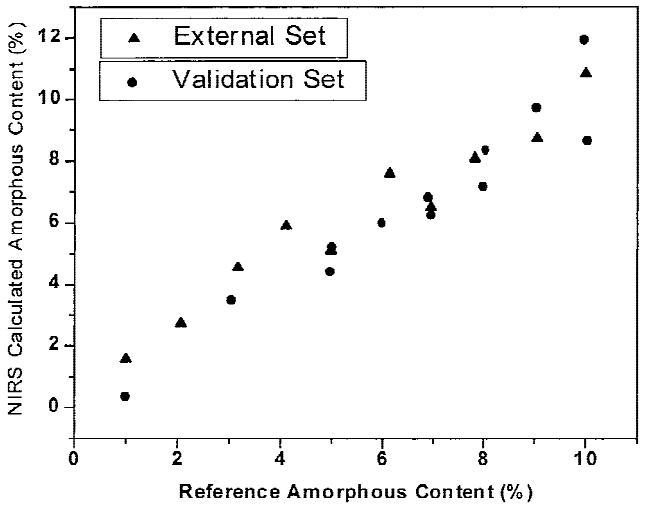

Fig. 1. Actual versus NIRS calculated amorphous content for the validation and external sets, using the calibration equation. For the validation set, a standard error of 0.84% w/w amorphous content was achieved, while the external set yielded a value of 0.99% w/w amorphous content.

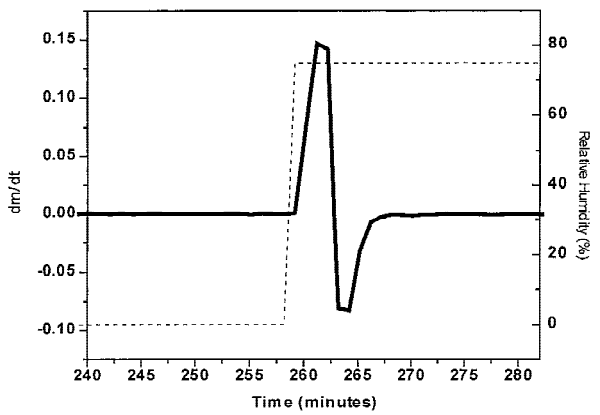

Fig. 3. Rate of change of mass (dm/dt) against time for a 5% w/w amorphous lactose sample. Before the mass increase, the sample is being exposed to a 0% RH drying step, and following the increase to 75% RH a mass increase and subsequent decrease is observed, in association with water sorption, plasticization, and crystallization. Integration of the peak above 0 will give a quantification of the water uptake, while integration of the peak below zero will quantify that water which is expelled. — = dm/dt; --- = RH (%).

the original amount of amorphous material within the sample (but not amorphous content expressed as percent w/w) (Fig. 4). Performing a linear regression on these data yielded a correlation of 0.929. The equation for the line of best fit was:

$$y = 0.330x - 0.102 \quad (3)$$

where y is the mass of water sorbed and x is the amorphous content. This line does not cross the x-axis at zero (intercept 0.309). Figure 4 shows that there seems to be a lower limit of detection at around 1 mg, where the technique would appear to be less accurate. As a result of this, samples with amorphous contents at or below this level do not yield a reliable result. There are a number of factors that may contribute to this. Primarily, at these very low amorphous contents, the water sorption response due to the crystalline portion of the sample may become significant. In addition, because quantification is reliant on an absolute original amorphous content, it does not reflect sample composition. For example, a 40-mg sample with an amorphous content of 8% w/w will contain the same amount of amorphous material as 80 mg of a 4% w/w sample. However the crystalline portion of the sample will sorb a certain, although small, amount of moisture, which may interfere with the results. As the amorphous content of the sample decreases, this error will have a greater impact on the accuracy of the results, so that at very low amorphous contents, accuracy is affected. In addition, as amorphous content decreases, errors arising from the preparation of the samples, such as the accuracy of weighing out small amounts of amorphous material, and the difficulty in achieving a uniform powder blend, will have a greater impact.

The area of the peak below zero of the same plot will represent the amount of water that is expelled from the sample. The mass as a function of time data showed that mass loss continued long after the dm/dt as a function of time peak had ended, indicating that this water loss continued at a steady rate after the initial fast expulsion. Despite the fact that the quantity of water that is desorbed is limited due to the formation of a partial hydrate, a correlation was seen between the amount of water desorbed and the original amorphous content. The assumption in this case is not that the total amount of desorbed water is being accounted for, but that some instantaneous process at the onset of crystallization involves the expulsion of water in a quantity directly proportional to the amount of amorphous lactose originally contained within the sample. These data are plotted in Fig. 5 and in this case the linear regression performed on the data gave a result of –0.899, implying that desorbed water is not as good an indicator of amorphous content as sorbed water. Samples below approximately 1 mg amorphous content are not seen to desorb detectable amounts of water, and therefore are not quantifiable by this method.

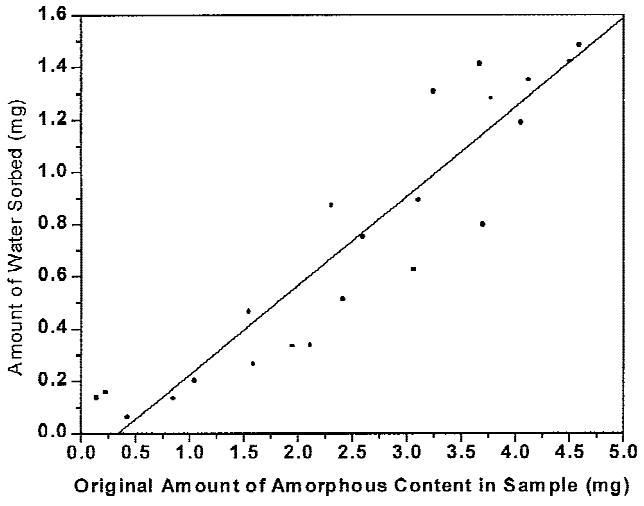

Fig. 4. Plot of water sorbed (water uptake) against the amount of amorphous material originally present within the sample. The line of best fit has a standard deviation of 0.17 mg, implying that this method is accurate to with approximately 0.2 mg of amorphous material with the original mass. Because the average sample mass used in this study was 40 mg, this corresponds to a level of accuracy of 0.5% w/w.

DISCUSSION

Initial sample preparation was carried out before any of the quantification work began, which means that some samples were used fresh, whereas others had been stored for several weeks before being studied, albeit under controlled conditions. The results obtained would indicate that this prolonged storage period did not prove problematic for either technique (because data for the same sample measured at the start and end of the process were the same within experimental error). Experimental design also meant that repeat runs on the same sample were carried out on different days. Any slight variation between those runs was within that which would be expected of both techniques. Amorphous lactose from two different spray-dried batches was also used and did not show any differences. Only one batch of crystalline lactose was used. Different batches of crystalline material are not expected to yield different results, because the amorphous lactose peaks are the key areas of interest. It can be seen, therefore, that the two experimental techniques allow reproducible data to be generated on different samples at different times.

As a further test of the reliability of NIRS, a third (external) batch of amorphous lactose was prepared, and from this, mixtures were formed with crystalline lactose. These new mixtures were used to test the existing calibrated equation that had been derived from NIRS (Fig. 1). This was done to

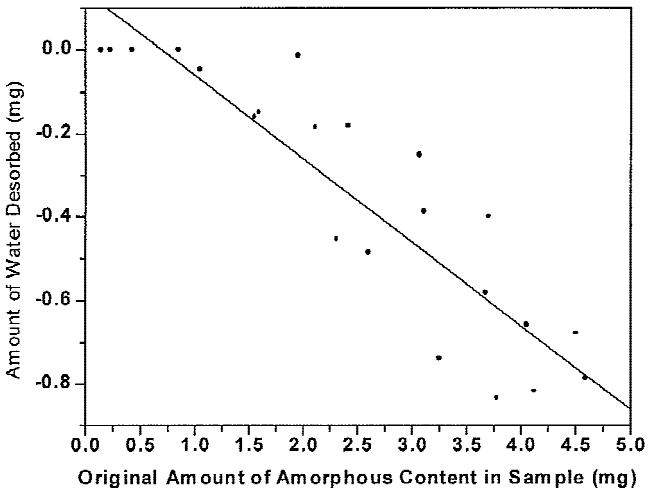

Fig. 5. The plot of mass of water expelled against the original amount of amorphous material.

ensure that the calibration equation was able to cope with entirely new samples. The SEP achieved by the technique was found to be 0.99 % w/w, thus demonstrating the validity of the equation. The DVS data on this new series of mixtures were identical to the original data within experimental error.

As already mentioned, DVS quantification is subject to a lower limit of reliability, which falls at approximately 1 mg amorphous content. The lower limit of quantification for this technique is therefore sample mass dependant. Assuming a sample mass of 50 mg, a 2% w/w amorphous content would be the lower limit. However, if that were increased to 150 mg, which is within the capability of the technique, then the best detection would be 0.7% w/w.

NIRS does not suffer the same lower limits of quantification. At a sample mass of 50 mg the technique is able to differentiate between samples of 0% and 1% w/w amorphous content. Additional work is required in order to establish the effect of sample size on NIR spectra, because it is likely that the powder bed contained within the sample pan allows some light to pass through it. Were the sample size to be increased such that the powder bed became deeper, this amount of light could be reduced, thus leading to a stronger reflectance signal being recorded.

CONCLUSIONS

It has been shown that NIRS and DVS are both capable of quantifying the amorphous content of lactose below 10% w/w. It is clear that at degrees of disorder as low as 1% w/w, either technique is capable of achieving a previously unattainable level of accuracy for quantification. Each technique has its own particular advantages. Recording an NIRS spectrum takes only a few seconds and complete recovery of the sample is also possible. DVS on the other hand provides more tangible information in the form of mass change. There are obvious advantages to having both techniques operating in combination because an immediate check of the NIR data can be obtained from the DVS sorption response. Furthermore, measuring the NIR data in the DVS ensures the complete control of the environment, and thus control of the water sorption peaks that would otherwise make a major contribution to the NIR response.

The NIRS-DVS data presented in this paper include spectra and mass changes for samples containing amorphous material originating from different spray-dried batches. This indicates that when a calibration has been established it is applicable to different batches.

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