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The novel combination of dynamic vapour sorption gravimetric analysis and near infra-red spectroscopy as a hyphenated technique

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Abstract

The novel combination of an environmental controlled gas flow microbalance (Dynamic Vapour Sorption, Surface Measurement Systems, UK) with a NIR spectrometer (Foss NIR Systems) is described. The study follows the gravimetric changes and the spectroscopic changes in the amorphous and crystalline states of lactose at 298 K. NIR spectra and gravimetric water sorption were recorded simultaneously for the same sample. Differentiation of the amorphous and crystalline states of lactose was possible from the evaluation of peak intensity and shifts in the known fingerprint regions of the NIR spectra, i.e. 1350–1510 and 1825–1975 nm which correspond to water changes, and 2075–2160 nm which tends to illustrate changes in the organic/structural backbone character. Gravimetric analysis confirmed that the amorphous lactose crystallised, as weight changes can be linked to structural changes. The combined technique maintains the high performance of the DVS microbalance for gravimetric analysis but also provides a preset, regulated and controllable environment for studies using NIR spectroscopy probes, which was previously not possible. The results obtained agree with accepted data, and therefore provide validation for the hyphenation technique. The use of the combined DVS-NIR instrument has indicated two new pieces of information, firstly the amorphous form loses some water before the crystallisation is detectable. This indicates that water desorption may precede crystallisation, rather than the other way around, and secondly, the sample has completed crystallisation before water desorption has ended.

Keywords: Near infra-red; Dynamic vapour sorption; Water sorption; Amorphous; Lactose; Crystalline

1. Introduction

Amorphous forms of drugs and excipients can be produced during processing and on storage they can revert to the thermodynamically stable crystalline forms (Briggner et al., 1994; Saleki-Gerhardt et al., 1994; Sebhatu et al., 1994). There

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will be changes in physical properties between different physical forms, potentially resulting in altered interactions with other phases of the formulation, e.g. dry powder inhalation formulations which require micronised drug to adsorb reversibly to a lactose carrier. Despite the importance of changes from the amorphous to the crystalline state, there are few techniques that can be used to detect the transitions.

The suitability of dynamic vapour sorption (DVS) for the study of pharmaceutical materials, particularly for the characterisation of amorphous lactose, has been well established (Buckton and Darcy, 1995, 1996, 1999). For the general routine analysis of pharmaceutical actives and excipients, near infra-red (NIR) spectroscopy is becoming a technique of choice (Wilson et al., 1997). The advantages of the technique are that it provides fast automatic acquisition of data with little sample preparation and no sample destruction. NIR spectroscopy has been used successfully to investigate the structural state of pharmaceutical materials, such as cellulose (Buckton et al., 1999) and the conversion of amorphous lactose to the crystalline state (Buckton et al., 1998).

When studying physical transitions in pharmaceutical powders it is often necessary to use many techniques in order to build a clear picture of what is happening. There are practical difficulties to consider when attempting to successfully transfer and correlate data from different instruments. Using amorphous lactose as an example, it is known that structural collapse will occur to different extents after varying times of exposure to a 50% relative humidity (RH) environment. Water is rapidly absorbed and desorbed by the amorphous material prior to collapse, however, following collapse sorption and desorption of water is a slow diffusion-controlled process. Some time after collapse occurs the amorphous material will crystallise with expulsion of the absorbed water, and retention of some water as a partial monohydrate. In essence the crystallisation due to water sorption is complex and its course will be affected by the water content and the rate of arrival or loss of absorbed water. In such a system it is often very difficult to relate the data generated from one instrument to those from another, due to uncertainties about whether the samples were exposed to exactly the same environmental conditions. To determine what change has occurred in a sample that gives rise to a mass change, it may be necessary to open the DVS instrument, remove a sample and run this in the NIR instrument. Of course opening the instrument and changing the environmental conditions is unsatisfactory and can easily affect the state of the material. There is much to be gained by linking two independent means of assessing changes in samples, in a way that allows comparable information to be gathered on the same sample simultaneously, eliminating the need to disturb the accurate control of the environment.

This study aims to reproduce the individually obtained DVS and NIR literature data (Buckton and Darcy, 1995, 1996, 1999; Buckton et al., 1998), validate the novel combined technique and provide an accurate examination of real time correlated data for gravimetric and spectroscopic changes, as an amorphous lactose sample crystallises. The concept of this hyphenated technique has been introduced previously (Lane and Buckton, 1999).

2. Materials and methods

The samples of amorphous lactose were prepared by spray drying an aqueous lactose solution (10 g per 100 ml) as described previously (Buckton and Darcy, 1995). This was then stored in vacuo over phosphorus pentoxide. The amorphous content was determined by crystallisation of a sample in a glass ampoule in the isothermal microcalorimeter (Thermal Activity Monitor — TAM, Thermometric) by exposure to 75% RH, giving an exothermic enthalpy of crystallisation of 50.6 J/g which is comparable to the literature value (Darcy and Buckton, 1998).

The combined DVS-NIR instrument is shown schematically in Fig. 1. A NIR probe was specially engineered by Foss NIR Systems, based on a fibre optic probe, but with the optics modified to allow collection of diffuse reflectance spectra.

The probe was connected to a NIRSystems 6500 spectrometer, and was housed in an adapted DVS (Surface Measurement Systems), so that the infrared source of the probe was always 3–4 mm below a flat glass sample pan within the DVS. This advance produces a specific technique that can correlate directly gravimetric and spectroscopic data in real time, under regulated and identical conditions. The pan contains the sample (up to 50 mg amorphous lactose) and the DVS instrument operates in the normal manner, at 298 K and over a preset humidity regimen. At a specified time interval the NIR instrument performs 32 scans, over approx. 40 s, in the wavelength range of 1100–2500 nm (9091–4000 cm^{-1}) and records the mean spectrum. It was initially uncertain whether the sample mass would be adequate to produce good quality diffuse reflectance NIR data, however, it proved to be perfectly satisfactory.

The DVS and the NIR can be synchronised to run together but are not linked by single software and therefore each instrument can still be operated independently.

In this study the DVS was set to perform an initial 0% RH drying step, then to switch to 75%

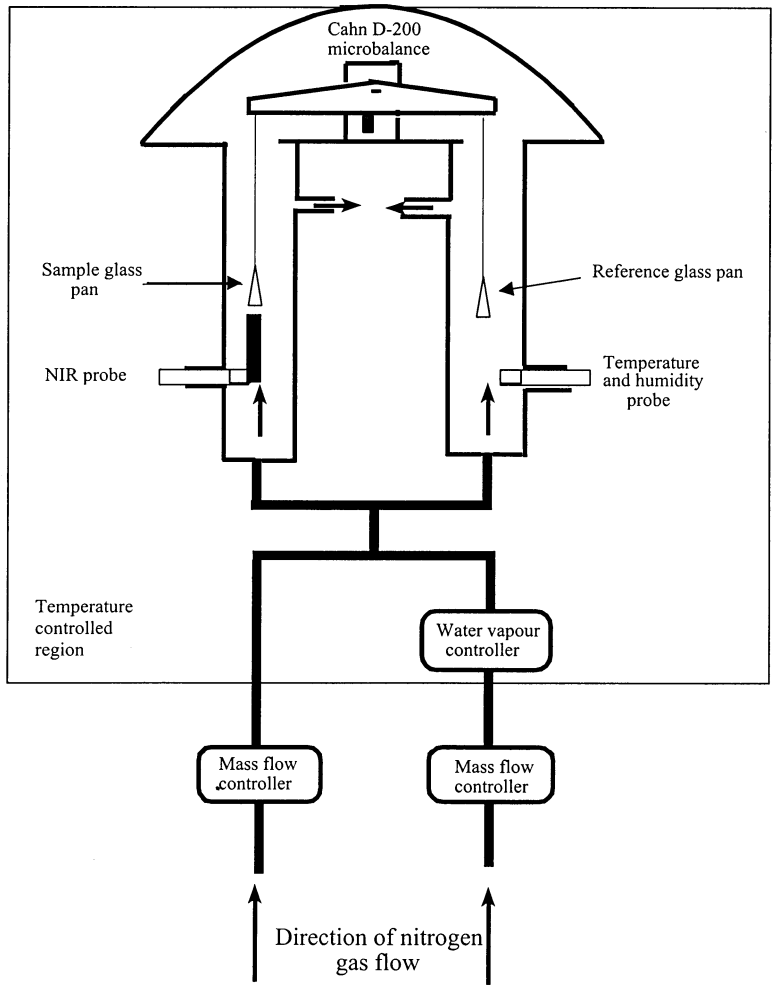

Fig. 1. Schematic representation of the combined DVS-NIR instrument.

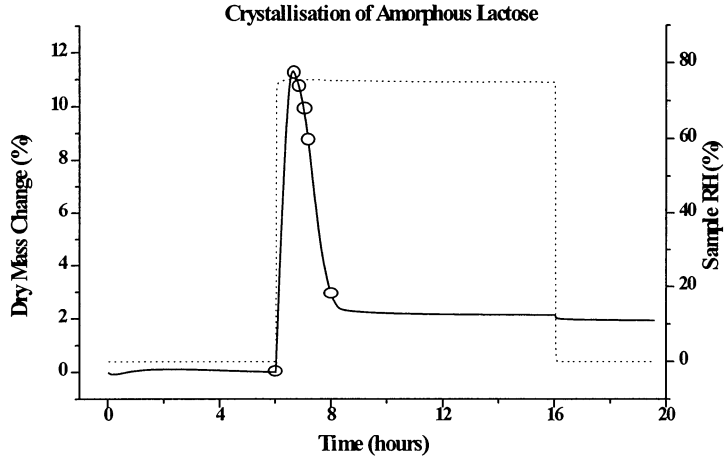

Fig. 2. Water sorption for amorphous lactose, exposed to 0% RH for 6 h, then 75% RH for 10 h then 0% RH for a further 4 h. Points marked indicate the collection times for the NIR spectra that are presented in the other figures.

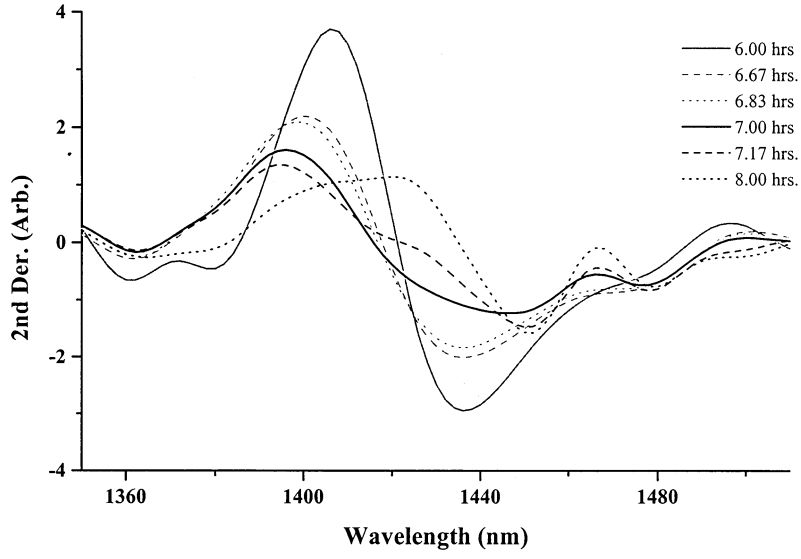

Fig. 3. Standard normal variance (SNV) second derivation NIR spectra in the region 1360–1500 nm showing the changes in the sample from 6 to 8 h (from dry to 2 h exposure to 75% RH).

RH to allow water uptake, structural collapse and crystallisation, and then a final 0% RH drying step. The NIR spectra were recorded every 600 s. Five repeat DVS runs were performed, each being essentially superimposed, and the data presented are typical responses, with NIR traces from the exact DVS run being shown.

3. Results and discussion

A typical DVS water sorption isotherm of the crystallisation of amorphous lactose from the combined NIR/DVS instrument is shown in Fig. 2. Also highlighted on Fig. 2 are the time points at which the NIR spectra, that are shown in Figs.

3–5, were collected. The DVS isotherm shows an initial drying of the lactose, i.e. a loss in mass and then a stable dry mass during the initial 6 h at 0% RH. The first 40 min of the 75% RH wetting step causes an increase in mass of 11.5%. From about 6.6 h (0.6 h of exposure to 75% RH) there is a large mass loss (9.0%) due to the crystallisation of the amorphous material and the resulting expul-

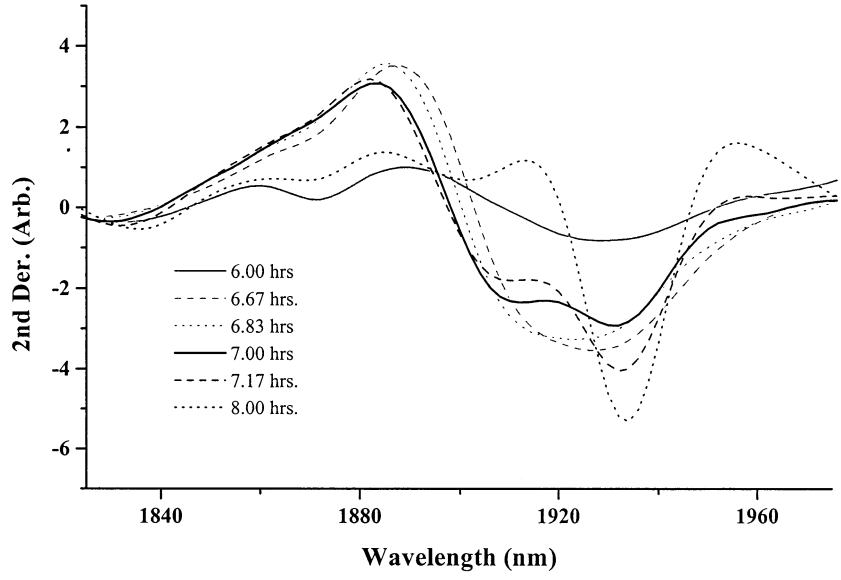

Fig. 4. SNV second derivation NIR spectra in the region 1840–1980 nm showing the changes in the sample from 6 to 8 h (from dry to 2 h exposure to 75% RH).

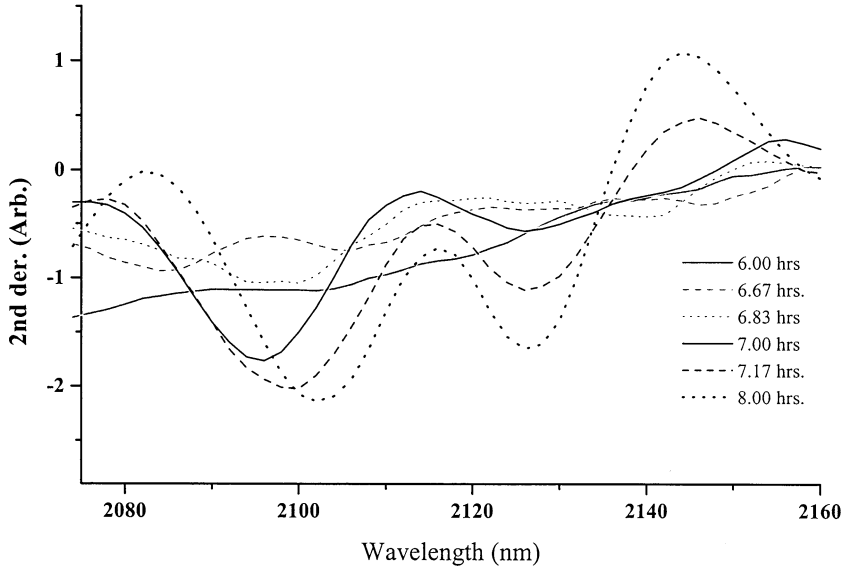

Fig. 5. SNV second derivation NIR spectra in the region 2080–2160 nm showing the changes in the sample from 6 to 8 h (from dry to 2 h exposure to 75% RH).

sion of absorbed water. Finally, 0% RH drying conditions were restored at 16 h, but an overall increase in mass of ~2.1% was recorded, due to the formation of crystalline lactose monohydrate. As lactose monohydrate contains 5% w/w water, a 2.1% weight retention would equate to less than half of the sample (ca 40%) having formed the monohydrate, with the rest of the sample being anhydrous, most probably β-lactose. These observations correspond favourably to amorphous lactose crystallisation data reported for the DVS technique (Buckton and Darcy, 1996), which demonstrates that the adaptation of the instrument has not altered the performance. By observing the DVS profile it is not possible to assert when collapse occurs, neither can the exact onset of crystallisation be determined, it can only be concluded that the crystallisation is underway when the weight gain stops and weight loss is seen. It is impractical to stop an experiment of this type to remove a sample for analysis by another technique, such as differential scanning calorimetry or powder X-ray diffraction, because the water-plasticised sample is very unstable and will change during removal and any subsequent test. Consequently, any information available from the NIR in situ will provide important proof of what happens to the sample at various stages during the water sorption process.

NIR is an area of infra-red spectroscopy that investigates vibrational phenomena at frequencies greater than 4000 cm^{-1} (wavelengths shorter than 2500 nm). NIR is the study of molecular bond absorptions that are harmonic overtones, or modulated ripples, of the fundamental vibrations that are found within the 'normal' infra-red range. Overtone bands are of lower energy (lower frequency or longer wavelength) and appear at integer multiples of the fundamental vibrations, e.g. overtones observed at 1436 nm (6964 cm^{-1}) and 1934 nm (5171 cm^{-1}) are the ripples of stronger fundamental vibrations found in the normal infra-red region at 718 nm (13 928 cm^{-1}) and 967 nm (10 342 cm^{-1}), respectively. The NIR spectra in this study have been normalised using standard normal variance (SNV) to decrease the detrimental effects of particle size changes that occur during an experiment. The second derivative of the spectra has been used as this helps to enhance differences in intensity and wavelength position, allowing genuine structural differences in the materials to be interpreted. In Figs. 3 and 4 the NIR regions corresponding to the first overtone –OH (~1450 nm) and the –OH deformation combination (~1940 nm) are shown respectively. In Fig. 3 a decrease in intensity of the derivatised spectra at 1436 nm is seen, but no shift in wavelength is observed due to the large uptake of water (~11.5% mass increase from the DVS). This intensity pattern is observed up to ~6.8 h (0.8 h exposure to 75% RH) in the NIR, after this point there is a general decrease in intensity and a shift in the wavelength to ~1450 nm. This corresponds to the onset of formation of the crystalline monohydrate, i.e. the –OH first overtone of lactose monohydrate, which is fully developed in the NIR by 8.0 h (2 h exposure to 75% RH). In Fig. 4 the structural change of collapsing lactose can be seen to develop between 6 and 6.8 h, being characterised by the increased intensity and shift to lower wavelengths of the peak at ca 1920 nm. Following the collapse the onset of the characteristic monohydrate peak (1934 nm) is seen and subsequently develops.

When the NIR spectra are correlated to the DVS weight changes (Fig. 2), it is clear that water begins to be expelled at an earlier point than the onset of the detectable formation of the monohydrate. Water loss begins as ~6.6 h, and continues after 8 h, whereas, the monohydrate starts to form between 6.8 and 7 h and has fully formed by 8 h when observed by NIR (spectra after 8 h are not shown). It is likely therefore that weight loss begins during collapse of the amorphous form and that weight loss continues after the sample has crystallised completely, due to slow diffusion of water away from less accessible regions of the sample. It has been shown previously (Buckton and Darcy, 1996) that collapsed amorphous lactose stored at 50% RH can be made to crystallise more rapidly if the humidity is reduced. In other words the reduction in water content in the collapsed form encourages crystallisation. In the current study it is suggested that the crystallisation occurs after the water content drops, even if the sample is maintained at elevated humidity. It is acknowledged that the detection sensitivity of

NIR for the presence of crystalline material in collapsed wet amorphous lactose is unknown, thus it is possible that very small amounts of crystallisation may precede water loss, but be undetectable by NIR. However, the evidence would indicate that crystallisation occurs during water desorption, probably starting after desorption, and certainly ending before the water desorption has finished.

In Fig. 5 the NIR region 2075–2160 nm is shown, peaks observed here relate to CH bonding as the crystal forms. A flat response is seen initially, which is typical of that for the amorphous form. This continues until 6.8 h (0.8 h exposure to 75% RH, and after the onset of water loss). The onset time for crystallisation in the 2075–2160 nm region was identical to that observed at the other wavelength regions and occurred between 6.8 and 7 h. At 7.0 h (1 h exposure to 75% RH) the alpha-monohydrate form can be detected by the presence of a single peak at 2095 nm. Curiously, by 8.0 h this peak disappears but develops into two other intensity peaks at $\sim$2103 and $\sim$2126 nm. β-Lactose has a characteristic NIR peaks at 2104 and 2126 nm due to O–H deformation and C–H stretching phenomena. The crystallised α-lactose is obviously still present in the sample, as the monohydrate peak is seen in the NIR spectra at 1932 nm and the gravimetric water retention in the sample indicates that *ca* 40% is the monohydrate. If this sample were stored at elevated humidity for a long time it would be expected to mutarotate entirely to the α-monohydrate form (Angberg et al., 1991, 1992a,b). However, it is clear that the NIR response for the β-form masks the response for the monohydrate in the proportions that are present in this sample. It perhaps suggests a lack of sensitivity of this NIR region for detecting the proportions of crystalline α and β lactose.

4. Conclusions

The combined instrument provides a real-time correlation of gravimetric behaviour and structural changes for amorphous lactose. Some interesting insights into the lactose system can be elucidated by the technique; the lactose material absorbs a significant amount of water ($\sim$11.5%) in the first 40 min, begins to expel water as it collapses for the next 10 min, and then appears to commence crystallisation. The NIR data show that the sample has finished crystallising before the water desorption process is completed. The DVS-NIR method thus provides a refined approach to continuous monitoring of physical transitions using weight change (moisture content) and spectroscopy to understand the transitions in the sample.

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