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The use of near infra-red spectroscopy to detect changes in the form of amorphous and crystalline lactose

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Abstract

The suitability of near infra-red spectroscopy (NIR) to follow changes in both the amorphous and crystalline state of lactose at room temperature was investigated. Amorphous lactose samples were stored in sealed glass jars with saturated salt solutions to control the relative humidity. NIR spectra were recorded after various periods of storage and the data related to calorimetric and thermo-gravimetric assessments of the physical form of the material. Differentiation between crystalline and amorphous states of lactose was found possible by studying the shape and magnitude of regions of the near infra-red spectrum corresponding to combination and first overtone stretching frequencies of water. It was possible to follow changes in the amorphous, the onset of crystallisation and the solid state transition from β- to α-lactose. NIR with benefits of being non-invasive, non-destructive and operating at room temperature, has been shown to be a valuable tool with which to assess changes in the physical form of lactose.

Keywords: Lactose; Amorphous; Crystalline; Polymorphism; Near infrared; DSC; Thermo-gravimetric analysis

1. Introduction

It is now acknowledged that the presence of any amorphous material in pharmaceutical formulations can have important consequences. Amorphous forms of many drugs and excipients can be produced during processing and can revert to the thermodynamically stable crystalline form on storage (Briggner et al., 1994; Saleki-Gerhardt

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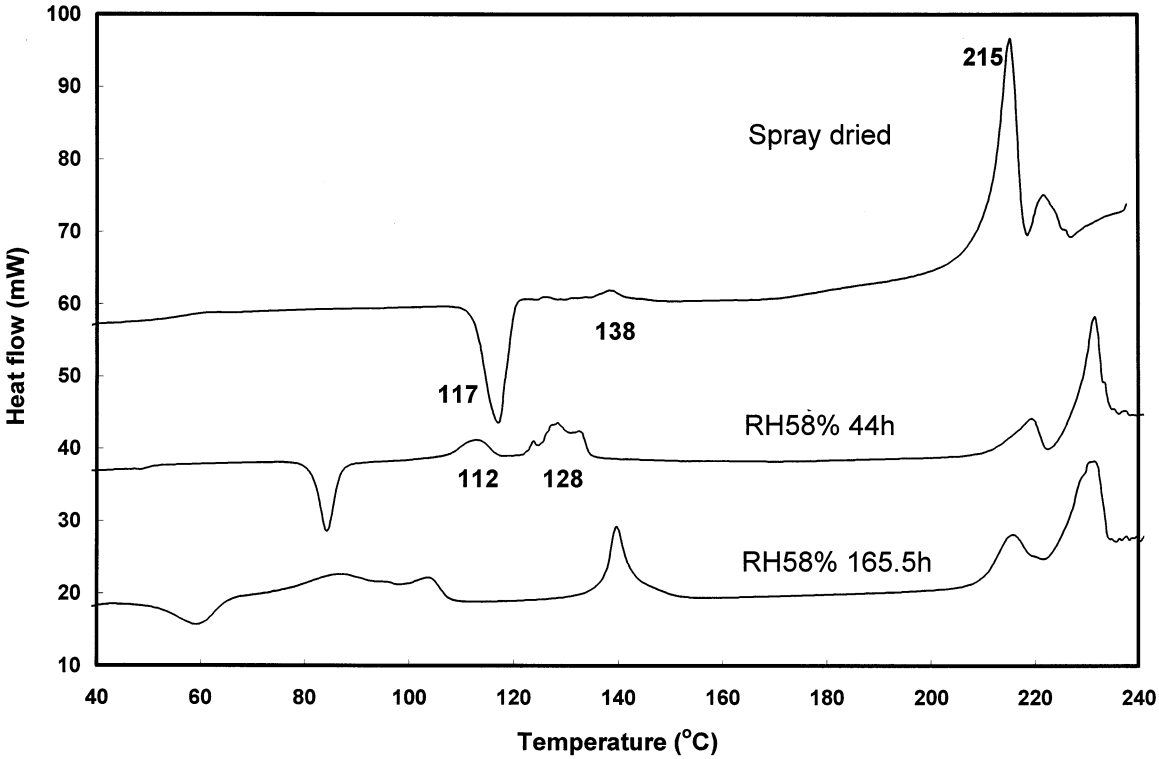

Fig. 1. DSC traces for spray dried lactose in the dry state, and after exposure to 58% RH for 44 and 165.5 h.

et al., 1994; Sebhatu et al., 1994; Buckton et al., 1995). The amorphous form will have different physical properties, and as such will interact with other phases in a different manner to that of the crystalline form. This can be important in many products, but not least in certain dry powder inhalations where micronised drugs must adsorb reversibly to a lactose carrier. An additional complication in systems which contain amorphous material is that the amorphous 'structure' can change in different conditions.

It has been recognised for many years that amorphous materials (if present in particulate, rather than thin film, form) can collapse when above their glass transition temperature, due to the inability of the rubbery material to support its own weight under gravity. Recently, Buckton and Darcy (1995) showed that amorphous lactose exhibited varying degrees of structural collapse depending upon the time for which it was held at 50% RH. It was noted (Buckton and Darcy, 1995) that water was rapidly absorbed and desorbed by the structure prior to collapse, but water sorption to and from the collapsed structure was slow and rate controlled by diffusion in the solid, rather than just by the external relative humidity (RH). It is reasonable to assume that the pre-collapsed and collapsed structures will behave differently in pharmaceutical products and further that the large quantities of water associated with the collapsed material may have a detrimental effect on products in which such material is included.

Despite the difficulties associated with the changes in physical form of materials, there are relatively few techniques which can readily detect changes in amorphous and crystalline form in real time. For example, powder X-ray diffraction is well suited to studies on changes in the crystalline state, but not to changes in the amorphous state. In this study near infra-red spectroscopy (NIR) has been used to follow the change in structure in amorphous lactose on exposure to different hu-

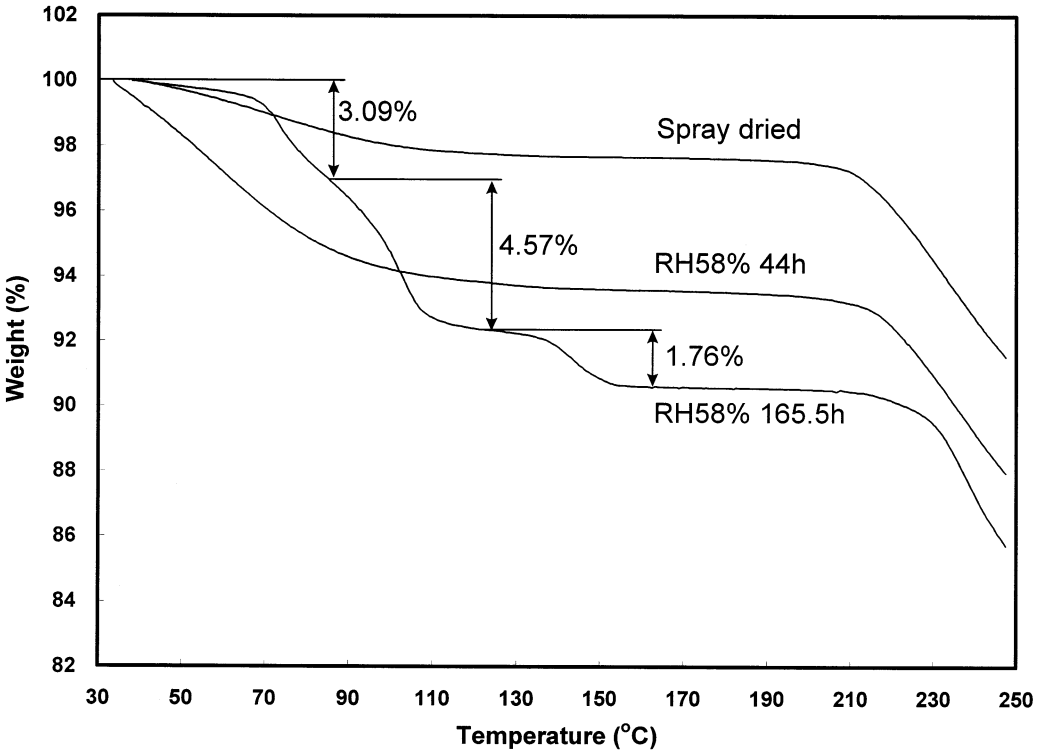

Fig. 2. Thermo-gravimetric analysis weight loss for the starting material and after storage at 58% RH for 44 and 165.5 h.

midities for different lengths of time. It is known that amorphous lactose will first collapse, trapping water in its mass, and then crystallise on exposure to 50% RH or more at 25°C. NIR was selected to investigate such changes as it is a rapid, non-invasive technique which requires minimal sample preparation, which has proved successful in studies of polymorphism (Aldridge et al., 1996) and water content of sugars (Kamat et al., 1989).

2. Materials and methods

Amorphous lactose was spray dried from solution (Briggner et al., 1994). The amorphous nature of the lactose was confirmed by exposure to 75% RH in an isothermal microcalorimeter (Briggner et al., 1994), yielding an area under the curve for crystallisation of 48 mJ/mg.

Samples (1 g usually, 2 g for the ageing study) of the amorphous lactose were placed into flat bottom clear glass jars with an air tight seal. A small tube of saturated salt solution was added and the jar was sealed, and stored in a temperature controlled environment (20°C). Periodically the jars were placed on the lens of the Rapid Content Analyser module attached to a NIRSystems 6500 spectrophotometer. The NIR instrument recorded the mean spectrum of 32 scans of each sample, (with a total scan time of approximately 40 s), over the wavelength region 1100–2500 nm. Replicate determinations of new samples were found to exhibit no significant differences from the original data sets.

Certain samples were taken immediately after recording the NIR spectra and analysed using differential scanning calorimetry (DSC) and thermo-gravimetrc analysis (TGA). The DSC experiments (Perkin Elmer DSC-7) were performed by loading ca 3 mg into non-hermetically sealed aluminium pans and heating from 40–240°C at 10°C/min, under a nitrogen flush. DSC calibration was with indium. The TGA (TA Instruments)

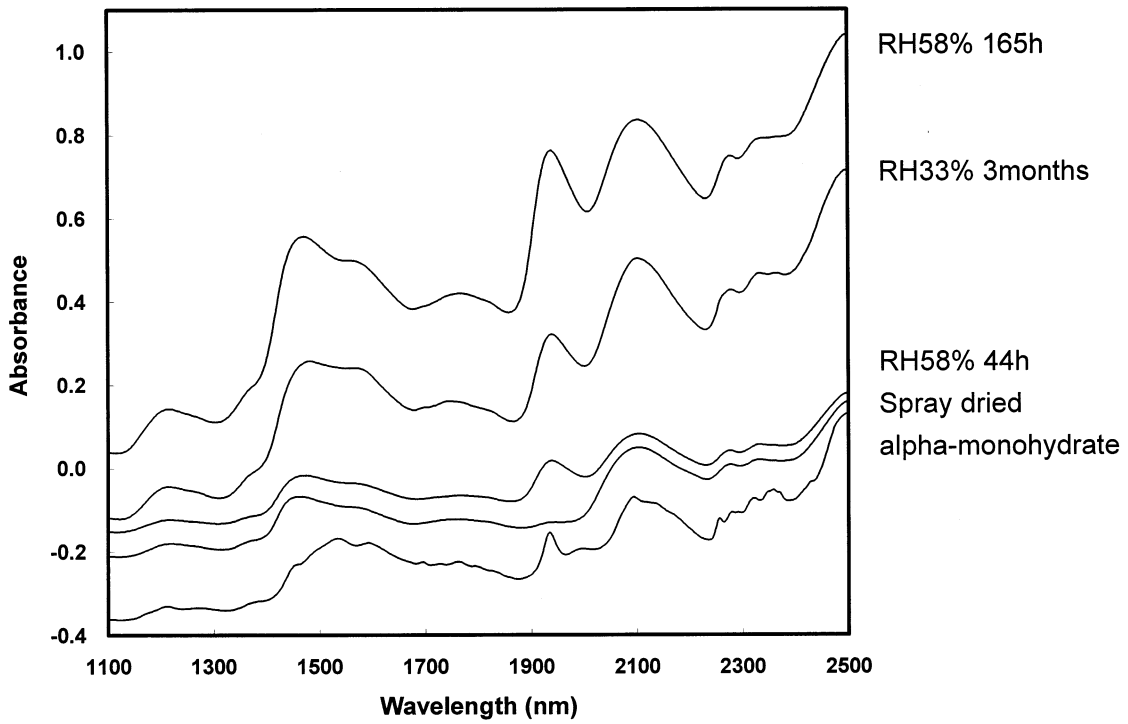

Fig. 3. NIR spectra for spray dried lactose, crystalline α-lactose monohydrate, and spray dried lactose after storage at different RH values for different durations.

was undertaken in open pans using ca 10 mg of sample over the same temperature range and with the same heating rate as for the DSC experiments.

3. Results and discussion

3.1. Differences in the amorphous state

The DSC data for the amorphous starting material are shown in Fig. 1. This can be explained as having a crystallisation exotherm at 117°C, followed by a small endotherm (water loss) at 138°C. At 215°C there is the α-lactose melt, followed by a small β-lactose melt. The low temperature for the crystallisation (literature values are normally at 185°C) is in keeping with the fact that some retained water has resulted in a lowering of the glass transition temperature. Also shown in Fig. 1 are selected data for samples which remain amorphous, despite having been exposed to elevated humidities for different periods of time (58% RH for 44 and 165.5 h). The sample which has been at 58% RH for 44 h has a much lower onset temperature for crystallisation than the original spray dried material, due to the water acting as a plasticiser. The onset of crystallisation (exotherm) is followed by a series of endotherms (main peaks at 112 and 128°C). These endotherms have been noted previously for collapsed amorphous structures, and represent the loss of water which has been trapped in the glassy structure. Thermogravimetric analysis (Fig. 2) confirms the fact that water is lost in these regions. Water which is lost in the region between 100–130°C is neither readily desorbable (i.e. it is not physically adsorbed water), nor is it hydrate water (which is lost at 150°C). As such these samples represent amorphous materials with different levels of entrapped water, with the entrapped water being a consequence of the collapsed structure. The sample which had been exposed to 58% RH for 165 h showed multiple peaks in the range 80–110°C, reflecting water loss from a collapsed amorphous

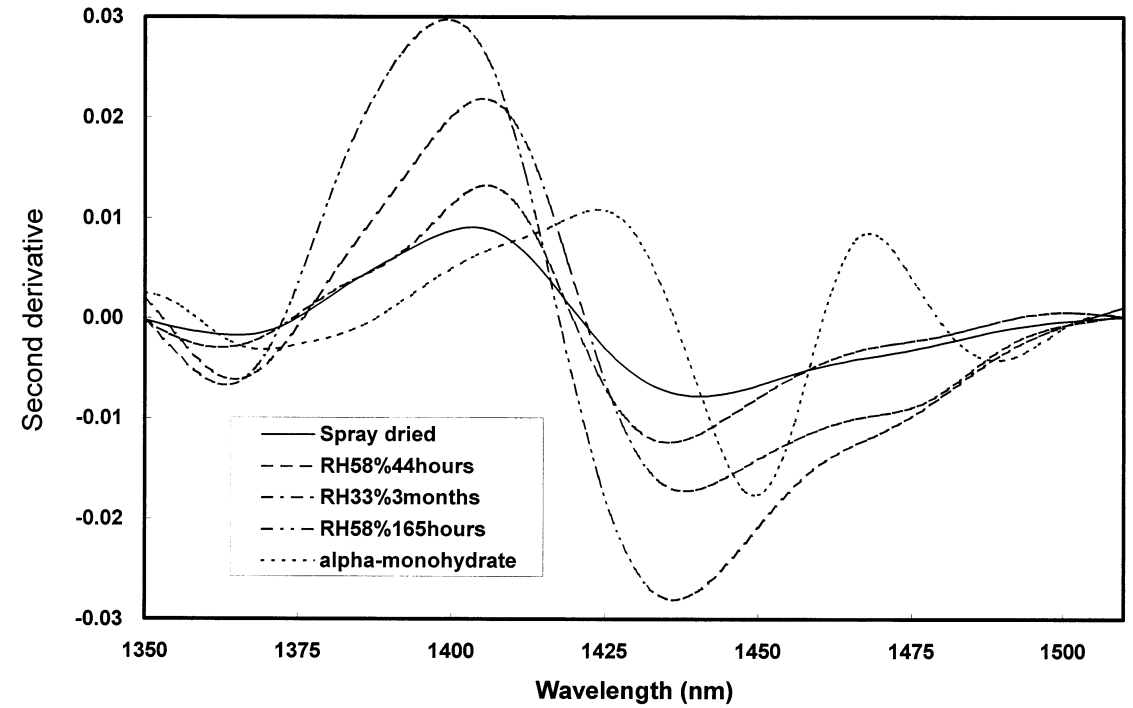

Fig. 4. Second derivative of the NR spectra in the region 1350–1510 nm for samples which remain amorphous.

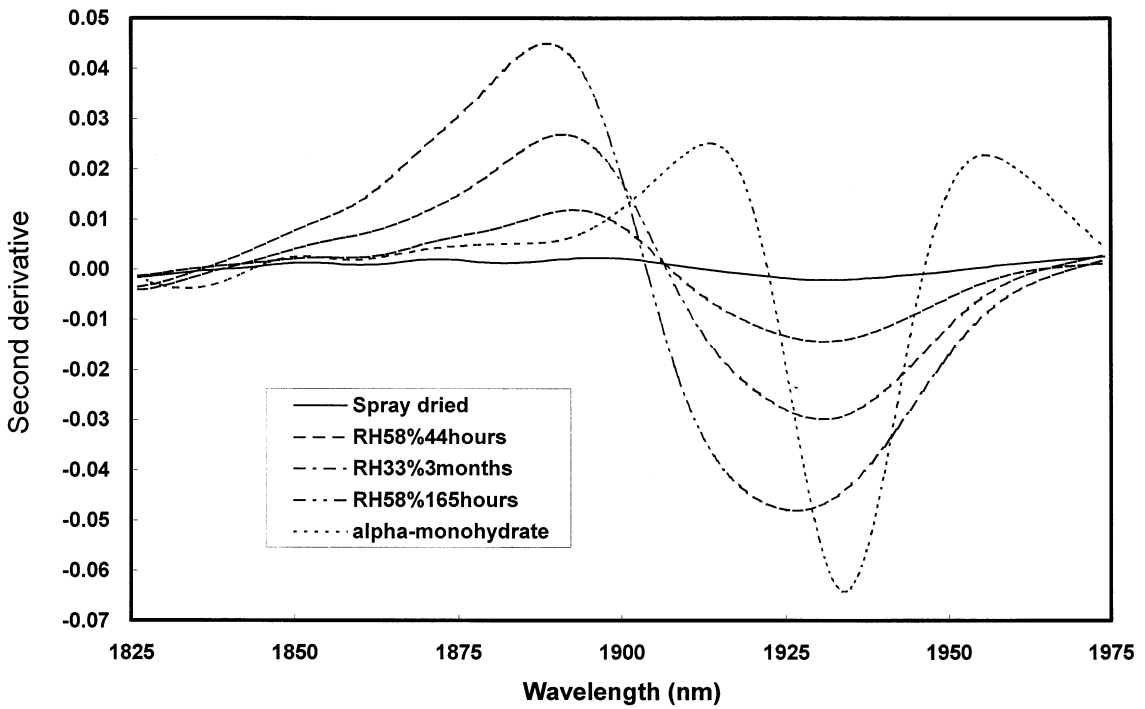

Fig. 5. Second derivative of the NIR spectra in the region 1825–1975 nm.

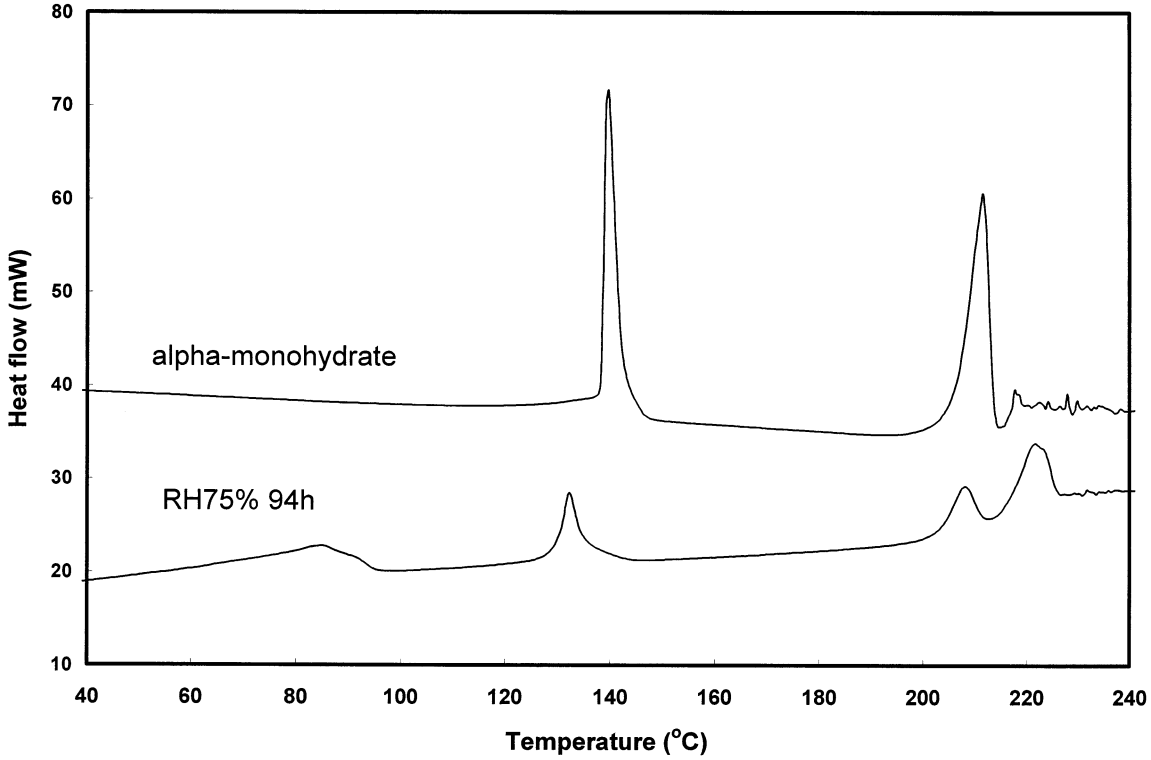

Fig. 6. DSC traces for α-lactose monohydrate and spray dried lactose after storage at 75% RH for 94 h.

structure, but there was also an extra peak at ca 140°C. Monohydrate loss is generally at ca 150°C, but it is likely that this could represent the onset of the formation of a partial crystal hydrate. The formation of a partial hydrate is supported by the TGA response (Fig. 2) for this sample which showed 1.76% water loss in the region of monohydrate water (less than the 5% that would constitute a stoichiometric hydrate). A difficulty with DSC is in deciding whether this region of partial hydrate existed in the sample before heating, or whether it formed as a consequence of the DSC run.

The NIR spectra for these samples are shown in Fig. 3 in comparison with crystalline α-lactose monohydrate, and a spray dried sample which had been stored at 20°C/33% RH for 3 months. An atmosphere of 33% RH does not lower the glass transition temperature (T_g) below room temperature, which was supported by the fact that the DSC and TGA traces for this sample (not shown) did not reveal the presence of extensive collapse, but simply a reduced onset of crystallisation (70°C), and an endotherm and water loss immediately above this crystallisation. The data in Fig. 3 show significant upward displacement from the spray dried sample less than 58%/44 h, less than 33%/3 months and less than 58%/165 h. These differences are a consequence of changes in size within the sample as water is absorbed and the particles swell, and ultimately fuse together following crystallisation. The NIR spectrum of a sample contains both physical and chemical information. The effect of particle size changes may be noted by a general offset of the baseline across the spectral region. Mathematically treating the data by use of second derivative function will result in the exclusion of the upward baseline shift. By this method a peak of negative displacement in the second derivative spectrum corresponds directly to a positively displaced peak in the original spectrum. Therefore, in these discussions the negatively displaced peaks are the ones of interest and are referred to as peaks (although they may

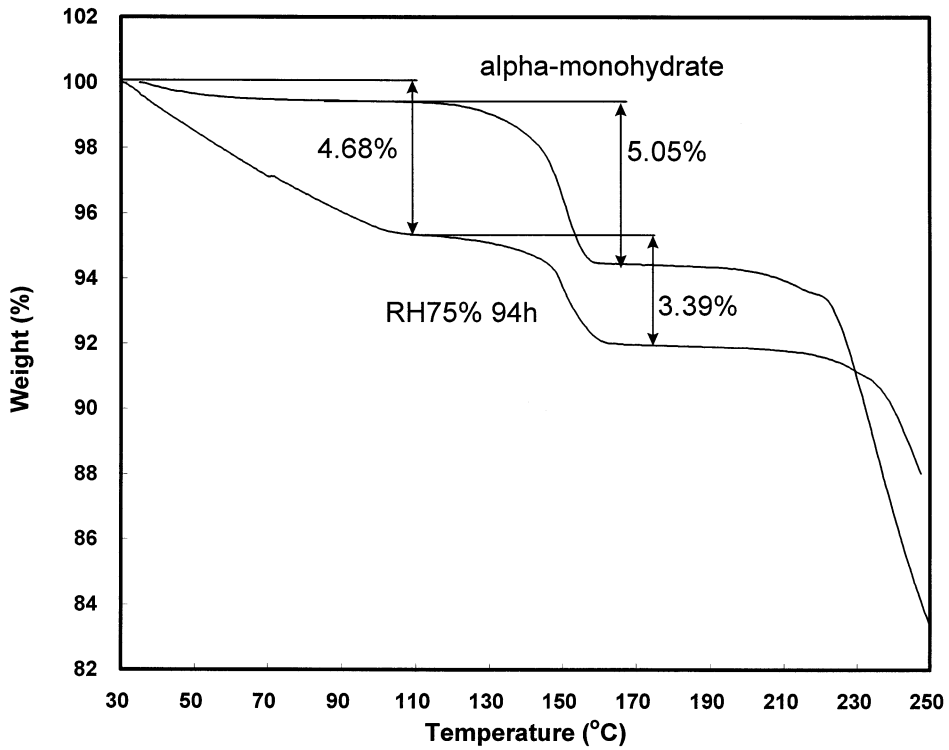

Fig. 7. Thermo-gravimetric analysis for α-lactose monohydrate and spray dried lactose after exposure to 75% RH for 94 h.

be considered as 'troughs'). Figs. 4 and 5 are appropriately scaled to highlight the regions in the NIR spectrum corresponding to the first overtone –OH (approx. 1450 nm) and the –OH deformation combination (approx. 1940 nm), respectively.

In Fig. 4 it can be seen that all the samples are significantly different to the spectrum of the monohydrate standard. The amorphous samples have a (negative—see above) peak for the water response at 1440 nm, which is significantly different to the position of the sharp monohydrate water peak at 1450 nm. The broad peak for absorbed water in the amorphous sample indicates a spread of energies of interaction, whereas the monohydrate water peak is typical of more uniform interaction. It can be seen that the peak for the absorbed water increases in size and displaces to lower wavelengths with increased exposure to humidity. The increase in size is due to the amount of water which has been absorbed, the shift in peak position indicates a change in median interaction energy. In Fig. 4 the sample stored at 58% RH for 165 h was found to have collapsed when examined by DSC and TGA, whereas the other amorphous samples were in the pre-collapsed state. It can be concluded from the fact that the collapsed sample has its water peak displaced to 1436 nm that, on average, the water is bound with a stronger interaction than for the pre-collapsed absorbed water. The collapse is due to the sample being plasticised by the water such that T_g falls below T, and hence the particles are unable to support themselves under gravity, thus they collapse into a densified glass structure. It is known that water can be released from the collapsed glass only by slow diffusion, whereas water desorption prior to collapse can be extremely rapid. These findings are in keeping with the shift of the NIR response to lower wavelengths.

In the region 1900–1950 nm (Fig. 5) it is possible to observe clear shifts in peaks as the sample takes up water in the amorphous region. A sharp (negative) peak at 1934 nm is seen for the monohydrate water, however, the amorphous samples

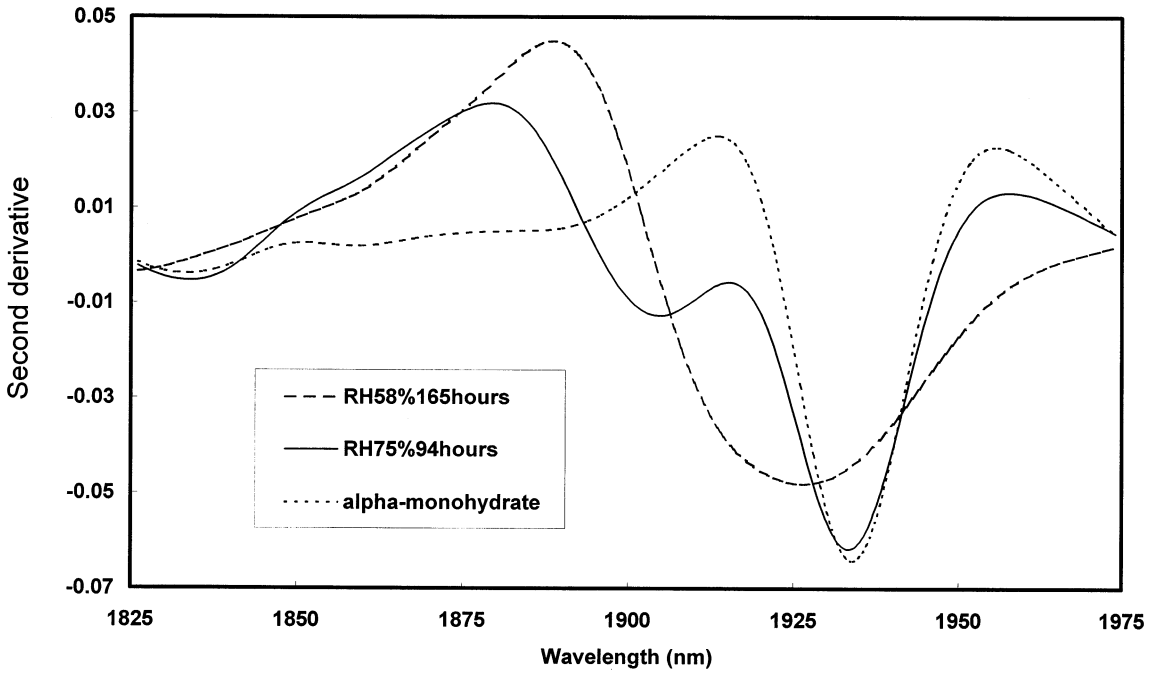

Fig. 8. Second derivative of the NIR spectra in the region 1825–1975 nm.

have a peak in this region which is displaced slightly to 1930 nm, and which continues to shift to lower wavelengths as the water uptake increases. The extent of shift in the position of this peak correlates with the arrival of the entrapped water that was driven off in the range 100–130°C (DSC/TGA), which is typical of collapsed amorphous lactose. It appears that the change in structure in the amorphous state can be followed for lactose by the change in the size and position of the peak in the 1900–1950 nm region.

The fact that the NIR spectra in Figs. 4 and 5 show no evidence of the characteristic monohydrate peaks would suggest that the partial monohydrate which is observed in the DSC and TGA responses for the 58% RH/165 h sample (Figs. 1 and 2) was in fact formed as a consequence of heating the sample (i.e. inducing crystallisation into a partial monohydrate).

Storage at higher humidities, or at 58% RH for longer times, results in the onset of crystallisation, however, in a sample of this size the crystallisation process is gradual. The DSC traces for samples as they crystallise are characterised by a gradual loss of the thermal events before the hydrate water loss at 150°C, and the increasing size of that hydrate water loss peak. Typical DSC and TGA responses for α-lactose monohydrate, and for a sample stored at 75% RH for 94 h are shown in Figs. 6 and 7. The second derivative NIR spectra for these samples are shown in Fig. 8. It can be seen that the peak in the region 1925–1940 nm indicates the extent of crystallisation in the sample. As crystallisation progresses the shape of the water peak becomes more sharply defined and the shift in wavelength from 1926 to 1934 nm, indicating a decrease in energy, confirms a change in the state of the water. As crystallisation begins, the peak for the remaining absorbed water shifts to a much higher interaction energy (peak at 1904 nm) indicating a clear transition. The difference between the responses for the 58%/165 h and 75%/94 h samples shows that the onset of crystallisation can be monitored in a non-invasive manner.

3.2. Following one sample as a function of time

Having shown that NIR can be a valuable tool for studies of changes in lactose structure, it is worthwhile testing this approach by following the

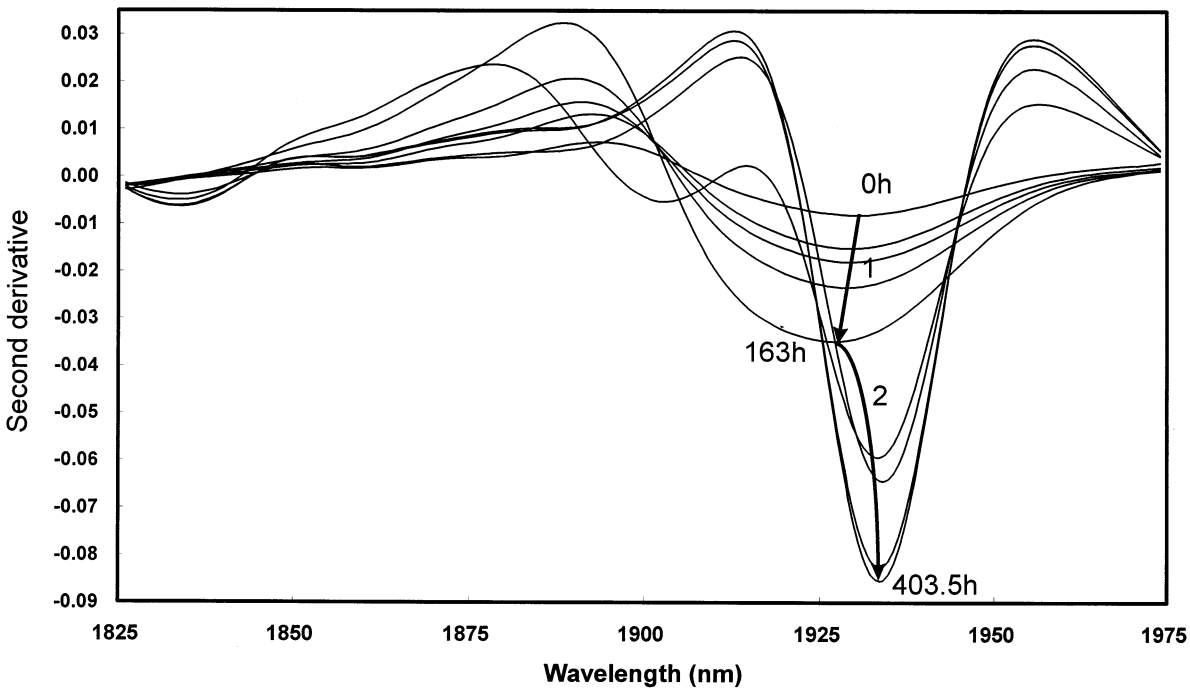

Fig. 9. Second derivative of the NIR spectra for the same spray dried lactose sample being measured as a function of time during exposure to 75% RH. The sample is amorphous—0–163 h, crystallisation occurs—163–403.5 h.

transitions in one sample at a defined RH (75%) over a period of time. Despite the presence of numerous spectra, it can be seen from Fig. 9 that firstly the water is absorbed causing the growth of the peak labelled 1. As crystallisation starts the hydrate water forms (peak labelled 2).

3.3. Proportions of α- and β-lactose

The DSC traces for the original spray dried sample show only an α-lactose melt (Fig. 1). As the sample moves towards the collapse region, the DSC data reveal predominantly β-lactose melts (Fig. 1), and finally when the sample has crystallised (before the DSC experiment was started) there are approximately equal α- and β-lactose melts (Fig. 6). It is however, hard to decide if these proportions of α- and β-lactose really existed before the DSC run, or whether they were formed during the DSC heating profile (as heating can induce mutarotation). The second derivative NIR spectra for a sample of β-lactose has characteristic (negative) peaks at 2104 and 2126 nm (Fig. 10), due to O–H deformation and C–H stretching. It can be seen that the amorphous material does not show these peaks (the spectra for the amorphous samples are those which had been exposed to 75% RH for 0–163 h and these are the spectra enclosed in the square bracket in Fig. 10), however, the samples which are just beginning to crystallise (186 h at 75% RH shown in Fig. 10) very clearly exhibit the characteristic peaks of the β-lactose spectral shape. With increased storage time at 75% RH (354.5 onwards) the characteristic β-lactose peaks are lost and the sample fits exactly to the spectral shape of the α-lactose standard. As such it can be concluded that the lactose first crystallised to be mostly crystalline anhydrous β-lactose, and then subsequently mutarotated in the solid state to crystalline α-lactose.

4. Conclusion

NIR can be used in real time to follow the changes in the amorphous state, the onset of

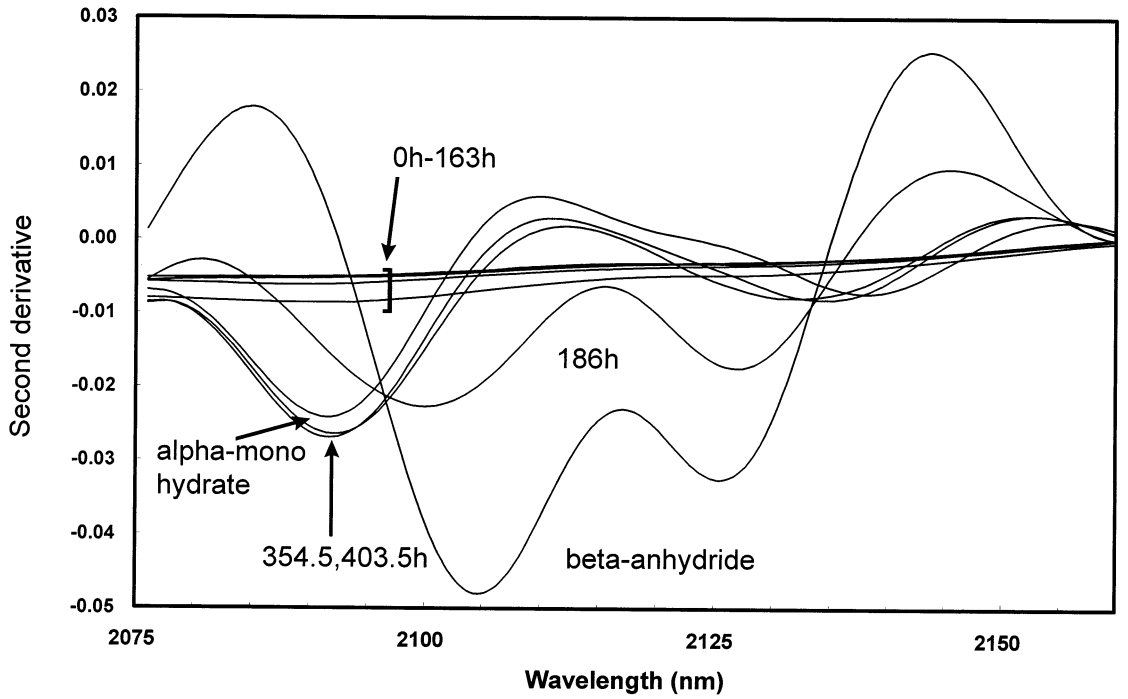

Fig. 10. Second derivative of the NIR spectra in the region 2075–2160 nm (C–H region) showing the absence of peaks for the amorphous samples (those bracketed are those exposed to 75% RH for 0–163 h). Then the presence of peaks which match those for the β-lactose standard at 186 h, then the transition of the peaks to match the α-lactose standard at 354.4 and 403.5 h.

crystallisation, and the changes between α- and β-lactose which accompany the onset of crystallisation. As such NIR has proved to be a valuable technique which can follow changes in both the amorphous and crystalline state for lactose. The fact that NIR is non-invasive and that spectra are measured at room temperature in a rapid and easy manner, makes it a very valuable tool to use in conjunction with other physical methods to study transitions in pharmaceutical materials. There are significant advantages in the use of the NIR technique, which is isothermal at room temperature, over the use of scanning calorimetry where crystallisation processes and transitions are induced due to heating, making it difficult to be certain of the properties of the starting material.

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