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Quantifying crystallisation rates of amorphous pharmaceuticals with dynamic mechanical analysis (DMA)

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ABSTRACT

One of the stability concerns for amorphous pharmaceuticals is phase transformation to a crystalline form. Since conversion from an amorphous matrix to a crystalline lattice should result in a change in mechanical modulus of the material dynamic mechanical analysis (DMA) offers potential as a stability-indicating assay for what are often complex formulations. Amorphous indomethacin glasses were used as model samples. Pockets made of a metal weave allowed the glass to be mounted in the instrument while ensuring exposure to RH. Crystallisation was manifest as an increase in the storage modulus signal with time. Conversion of the data to fraction crystallisation allowed quantitative determination of the rate and mechanism of crystallisation by application of the Urbanovici–Segal model. Rates of crystallisation were seen to increase with temperature and humidity while temperature and humidity affected the mechanism of crystallisation. High temperature and humidity resulted in three dimensional crystal growth. Reducing the humidity caused a switch in mechanism to growth from edges. Reducing temperature resulted in a mixed mechanism of growth from surfaces and edges. The DMA was also sensitive to crystallisation of phenobarbital sodium formulated in an oral film, but quantitative analysis was not possible as the onset of crystallisation was not recorded.

1. Introduction

Formulation of an active principal in an amorphous form is an attractive way to improve bioavailability of poorly soluble compounds because the lack of any crystal structure leads to faster dissolution and higher solubility. Usually polymeric excipients are included to create solid amorphous dispersions, an overarching term that describes both solid suspensions (amorphous drug particles dispersed in the amorphous polymeric matrix) and solid solutions (drug molecules dispersed in the amorphous polymeric matrix). Examples of marketed products formulated in this way include Sporanox™ (itraconazole, formulated as a solid solution on sugar granules), Nurofen® meltlets (ibuprofen, fast-dissolving oral tablets) and Ora-film™ strips (benzocaine, fast-dissolving oral film).

In addition to the usual stability concerns (chemical stability of the active and/or interaction with excipients) amorphous materials are not at a position of thermodynamic equilibrium, possessing an amount of excess energy over any available crystalline arrangements. With time (usually accelerated with an increase in temperature and/or relative humidity, RH) the molecules within the amorphous matrix will begin to orient themselves in more favourable positions, lowering the overall energy of the system. Initially this results in relaxation of the system and ultimately conversion to a crystalline form will occur. Given that product performance is contingent upon the system being amorphous, conversion to a crystalline form is likely to cause an unacceptably high reduction in bioavailability. Where products are formulated to be amorphous then assays must be designed to quantify stability with respect to temperature and humidity. In particular, pharmacopoeial standards for stability-indicating assays for oral films are lacking, which is surprising given the high concentration of (often volatile) plasticisers that they contain.

Stability in this case refers to physical form, rather than chemical composition, so conventional stability-indicating assays (such as HPLC, TLC or UV) are not applicable. Rather, assays must be developed based on techniques that measure a change in physical property. Calorimetry is an obvious choice, because there will be evolution of heat with either relaxation or crystallisation. Differential scanning calorimetry is widely used to study relaxation of glasses (Kawakami and Pikal, 2005) but is rather limited in its application to formulated systems because the sample size (5–10 mg) means the amount of active principal can be very low. Isothermal calorimetry is perhaps a better option (Liu et al., 2002), and has been

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shown to be able to monitor crystallisation in oral films (Gaisford et al., 2009) but the heat change is measured as the crystallisation proceeds in real-time, so the magnitude of the power signal can be very small.

Since changes in physical form usually occur with a concomitant change in mechanical modulus, an alternative technique is dynamic mechanical analysis (DMA). DMA measures the viscoelastic modulus of a material as a function of time, temperature and/or RH. Typically DMA is used to characterise plastics and novel composite materials, but its use in the pharmaceutical arena has been growing, with recent application to glass transitions of powders (Royall et al., 2005; Mahlin et al., 2009), hot-melt extrudates (Yang et al., 2011), pellets (Bashaiwoldu et al., 2004) and enteric polymer films (Fadda et al., 2008, 2010; Perfetti et al., 2010). These studies have all focussed on determination of either mechanical moduli or glass transition temperatures. The specific aim of this work is to develop a method of analysis that allows determination of the isothermal rate of crystallisation and hence the use of DMA as a stability-indicating assay. The method is applied to both a pharmaceutical glass (one phase) and a simple oral film formulation (considered here as two phases).

2. Materials and methods

Crystalline indomethacin (99%+), polyvinyl alcohol (PVA, 98% hydrolysed, molecular weight 13–23,000), carboxymethyl cellulose sodium salt (NaCMC, USP grade), phenobarbital sodium (NaPHB, 99%+) and magnesium chloride (ALR) were purchased from Sigma–Aldrich (UK). Glycerol (bidistilled, 99.5%+, Analar Normapur) was purchased from VWR (UK). All materials were used as received. The metal mesh (Code HT25) was purchased from United Wire Ltd. (UK). The mesh is woven in a Dutch Twill pattern (1 warp to 2 weft wire), has a porosity of 47% and a nominal particle retention size of 25 μm (Fig. 1).

2.1. Preparation of indomethacin glasses

Indomethacin glasses were prepared by melting indomethacin (typically 0.3–0.4 g) in a silicone mould on a hot-plate. The mould was 1 cm^2 and 2 mm deep. Once molten the sample was removed from the hot-plate and allowed to cool to ambient temperature. Care was taken to ensure no air-bubbles were present. Once cold the glass was carefully removed from the mould (Fig. 2) and stored in a fridge (4 ± 2 °C). Glasses were used within 48 h of preparation.

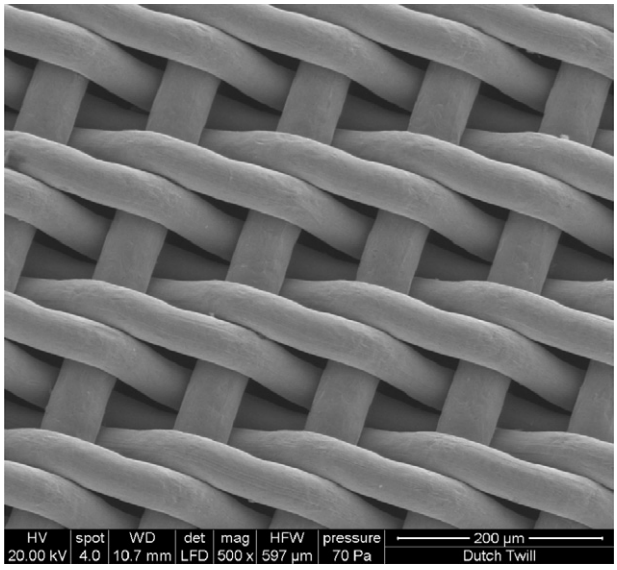

Fig. 1. SEM image of the metal mesh showing the Dutch twill weave pattern.

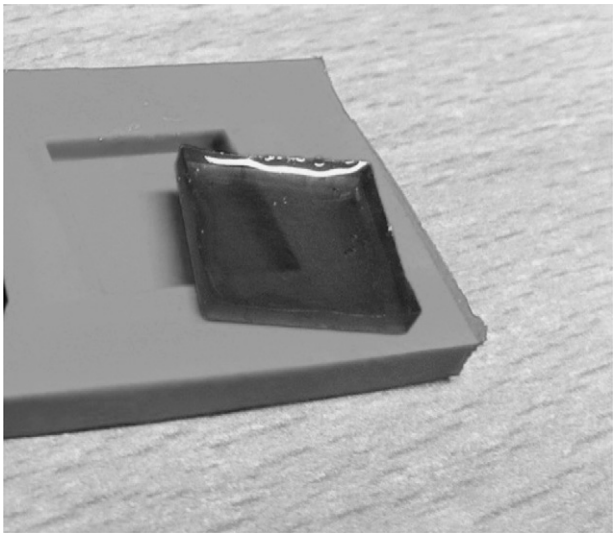

Fig. 2. A moulded square of indomethacin glass.

2.2. Preparation of oral films

Oral films were prepared by solvent casting. PVA (3.75 g) was added to water (150 mL) and heated to 80 °C. Following dissolution NaCMC (3.75 g) was added and the mixture was allowed to cool to room temperature with stirring. Glycerol (1.8 g) and NaPHB (286.3 mg) were added and the mixture left to stir for a further hour. The solution (15 mL) was poured onto a Teflon mould (circular with a diameter of 9 cm) and dried overnight in an oven (30 °C). Film strips for DMA analysis were cut from the cast film (typically 0.6 cm × 2 cm).

2.3. DMA of indomethacin glasses

All DMA measurements were performed with a Q800 DMA (TA Instruments Ltd.) equipped with an RH accessory. The indomethacin glasses were too small and fragile to mount in the DMA clamp directly. Powder-pockets allow the study of self-supporting materials by DMA (Royall et al., 2005; Mahlin et al., 2009) but could not be used here because exposure of the sample to RH was essential. Instead samples were housed in a metal pocket formed from the metal mesh. The mesh was cut into a strip 2 cm × 5 cm and then folded in half along its length (Fig. 3). The mesh was loaded into a dual-cantilever clamp and the 2 void

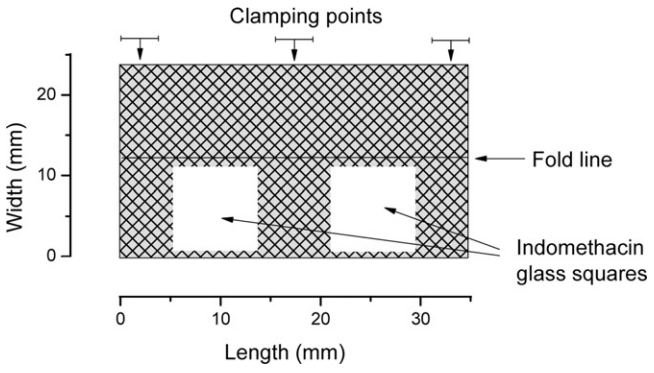

Fig. 3. Schematic diagram of the metal weave sample holder and position of the indomethacin glass squares.

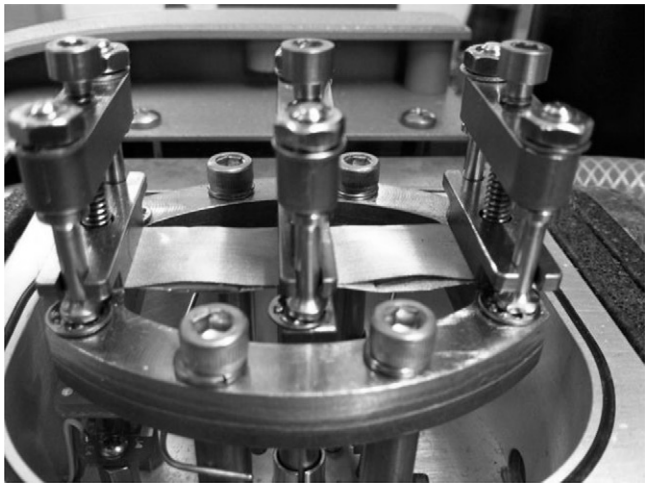

Fig. 4. The metal mesh mounted in the dual cantilever clamp with the indomethacin glasses in the void spaces.

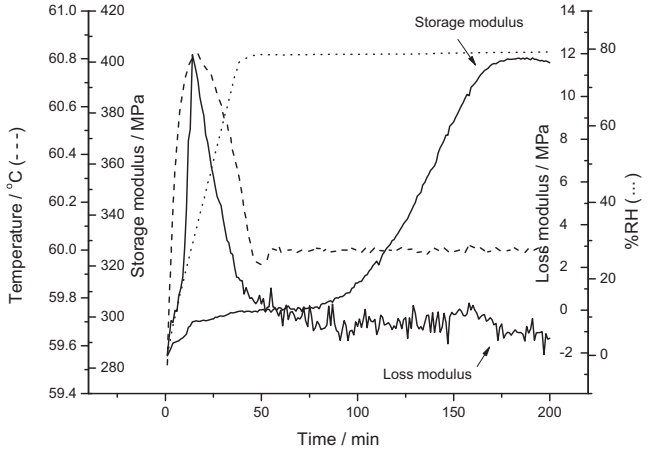

Fig. 5. DMA data for crystallisation of an indomethacin glass held at 60 °C and 75% RH.

spaces between the clamping points were each filled with an indomethacin glass (Fig. 4). The clamps were tightened to 2 in lb. To ensure the metal mesh exposed the sample to humidity, its performance was checked using the deliquescence point of magnesium chloride as a reference material (expected 32.8%, measured 31.29%). Experiments were performed at an oscillating frequency of 10 Hz, 15 μm amplitude.

2.4. DMA of oral films

Oral films were loaded directly into the film clamp. Experiments were performed with the film under tension (to prevent buckling). A static pre-load force (0.01 N) was applied to the sample prior to the dynamic oscillating force. During measurement the instrument was programmed to maintain the static load at 125% of the force required to oscillate the sample. It is important that the film remained in its linear viscoelastic region during measurement (to ensure that the properties observed were independent of the deformation applied and truly reflected molecular motions) and so experiments were recorded maintaining constant strain. Generally, for thin polymer films, linear viscoelastic behaviour can be assured with a strain less than 0.1% and so this limit was used. Experiments were performed at an oscillating frequency of 1 Hz.

3. Results and discussion

DMA applies an oscillatory stress to a sample (and measures the corresponding strain). In the case where a material is a Hookean solid (such as a metal) perfectly elastic behaviour will be seen (i.e. the strain will be exactly in phase with the applied stress). Typical pharmaceutical samples are not perfectly elastic, however, but viscoelastic and so the measured strain will lag behind (i.e. be out of phase with) the applied stress by some angle, δ, the maximum phase lag being 90°. The overall modulus is thus a complex modulus, comprising an in phase (E' or storage modulus, reflecting elastic behaviour) and an out of phase (E'' or loss modulus, reflecting viscous behaviour) component.

Fig. 5 shows the loss modulus and storage modulus data for an indomethacin glass held at 60 °C and 75% RH. Upon removal from the instrument the indomethacin was seen to be crystalline (Fig. 6) and so, in the absence of any other processes occurring in the sample, the data reflect changes in the viscoelastic properties of the sample as crystallisation progresses. All samples crystallised to the α-polymorph (melting point by DSC, ca. 155 °C, data not shown) irrespective of the temperature and/or humidity of the experiment. This is in accordance with the previous observations that the α-polymorph forms at temperatures of 50 °C and above (Andronis and Zografi, 2000) and RHs of 56% and above (Andronis et al., 1997). Following attainment of temperature and humidity equilibrium (ca. 50 min) the loss modulus signal was seen to remain fairly constant. The noise in the signal reflects the small value of the loss modulus, relative to the storage modulus. This is not surprising, since the metal mesh is comprised of a perfectly elastic metal (loss modulus of zero) and the indomethacin, while a glass initially, is a high viscosity solid (and so has a small loss modulus value), although Andronis and Zografi (1997) found the loss modulus to be an indicator of molecular mobility in glassy indomethacin. Here the storage modulus value was seen to increase with time, reflecting the increasingly elastic behaviour of the indomethacin as it transformed to a totally crystalline phase.

Note that although the storage modulus is thus a suitable signal for following crystallisation, determination of its absolute value requires knowledge of the geometry of the sample. Here the indomethacin glasses, although cast from a mould, varied slightly in terms of thickness. Moreover, the volume of a crystalline matrix is by definition smaller than that of an amorphous matrix and so shrinkage occurred during the experiment (apparent from Fig. 6). The orientation of the metal weave is also important, because if its directional pattern. Thus absolute moduli could not be determined. However, *changes* in modulus, for a given sample, are a quantitative indicator of change during an isothermal measurement and it is on this basis that the analysis method is predicated.

Fig. 6. Crystalline indomethacin post-analysis in the DMA.

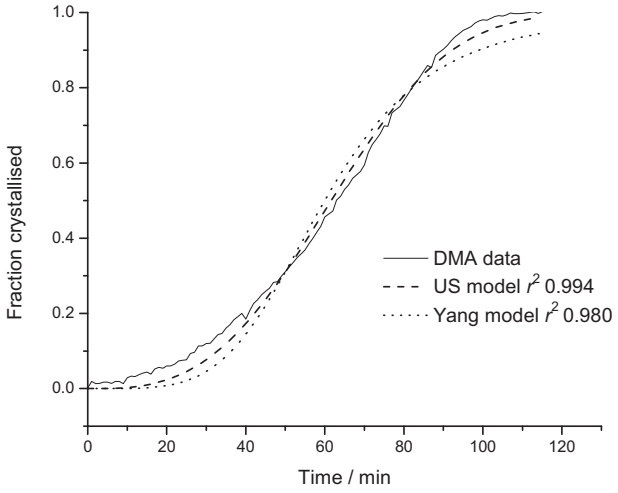

Fig. 7. Crystallisation isotherm for indomethacin glass at 60 °C, 75% RH and the fits obtained to the Urbanovici–Segal (US) and Yang models.

Quantitative analysis of the rate of crystallisation usually starts with conversion of the experimental data to fraction crystallised with time. Let the storage modulus of the sample initially (i.e. when it is a glass) be E'_0 and the final storage modulus of the sample (i.e. when it is crystalline) be E'_∞. Both values can be determined from the DMA data as shown in Fig. 5. Then:

$$\text{Fraction crystallised} = \theta_t = \frac{E' - E'_0}{E'_\infty} \quad (1)$$

The resulting isotherm is shown in Fig. 7. Analysis may then proceed using any of the models available in the literature. The starting point is usually that of the Avrami model (Avrami, 1939):

$$\theta_t = 1 - \exp(-kt^n)$$

where k is the rate constant and n is the Avrami exponent, the value of which defines the mechanism of crystallisation (a value of 3 represents crystal growth in three dimensions, assuming pre-existing nuclei, a value of 2 indicates crystal nucleation and growth from edges and a value of 1 represents nucleation and crystal growth from surfaces. Non-integer values reflect mixed mechanisms (Cahn, 1956)).

The Avrami model has been widely discussed and is generally recognised to break down as crystallisation approaches completion. This is because the model assumes both a constant rate of crystal growth and a constant increase in crystal size; as such it does not account for crystal growth impingement as crystallisation reaches completion. Many derivatives of the Avrami model are available; in an earlier study of crystallisation of indomethacin from polymer films Gaisford et al. (2009) applied three such models and determined that the Urbanovici–Segal version best described the data:

$$\theta_t = 1 - [1 + (r+1)(k_{us}t)^{n_{us}}]^{1/(1-r)} \quad (3)$$

where K_{us} is the Urbanovici–Segal rate constant, n_{us} is the Urbanovici–Segal exponent and r is a parameter that satisfies the condition $r > 0$. When $r = 1$, Eq. (3) reduces to the Avrami equation. Supaphol (2001) notes that the physical meaning of the term r is unclear, and it may be the case that it is simply a parameter that determines the degree of deviation of the Urbanovici–Segal model from the Avrami model.

Table 1
Summary of the kinetic parameters obtained by fitting the crystallisation isotherms to the Urbanovici–Segal model with least squares minimisation.

Storage conditions	k (min^{-1})	n	r
60 °C, 50% RH	4.4×10^{-4}	2.04	1.01
60 °C, 60% RH	5.5×10^{-4}	2.06	1.01
60 °C, 75% RH	2.6×10^{-3}	3.00	1.01
50 °C, 75% RH	1.08×10^{-4}	1.45	1.01

More recently, Yang et al. (2010) discussed a new approach to kinetic modelling of amorphous solid dispersions and derived the following general form of the Avrami model:

$$\theta_t = 1 - \frac{1}{1 + kt^n} \quad (4)$$

The crystallisation isotherm was fitted to both Eqs. (3) and (4) by least squares minimisation (Fig. 7). It is apparent that the fits were good, as indicated by the correlation coefficients of 0.994 (Urbanovici–Segal) and 0.980 (Yang), although both models tended to break down as crystallisation approaches completion. This is unlikely to reflect the impact of crystal growth impingement, since both models account for this, but may be related to the shrinkage of the sample because, as noted earlier, sample geometry will affect the measured modulus and is not accounted for in either model. The models also produced different rate constants (Urbanovici–Segal 0.0026 min^{-1}, Yang 0.017 min^{-1}) and Avrami indices (Urbanovici–Segal 3.0, Yang 4.4). The Urbanovici–Segal model was found to give the better fit to the data in this case, and so was used for all subsequent analyses.

The rate of crystallisation should be dependent upon RH and temperature, an increase in either increasing the rate. Such isotherms are shown for glasses held at different RHs in Fig. 8 and at different temperatures in Fig. 9. Also shown in Figs. 8 and 9 are the fit lines obtained from the Urbanovici–Segal model. Note that prior to fitting the data to the model the time at which crystallisation was first observed was set equal to zero. The values for the parameters obtained are given in Table 1.

The rate constants increased with temperature and/or RH as qualitatively indicated by the positions of the isotherms in Figs. 8 and 9. No directly comparable literature data are available, although Andronis and Zografi (2000) showed crystallisation isotherms for indomethacin under 0% RH at 50 and 60 °C (and

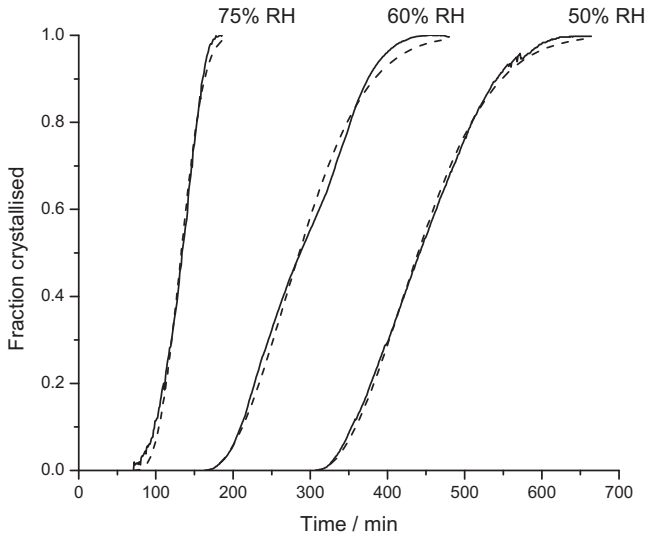

Fig. 8. Fraction crystallised versus time plots for indomethacin glasses at 60 °C and varying relative humidities. Experimental DMA data (solid line) and fit to US model (dotted line).

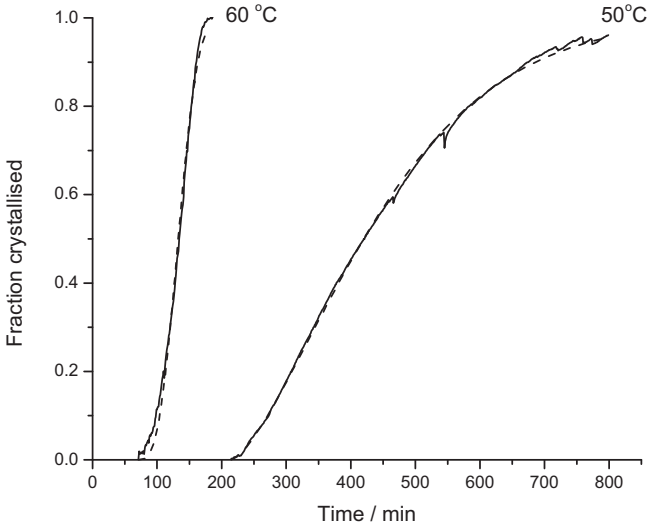

Fig. 9. Fraction crystallised versus time plots for indomethacin glasses held at 75% RH and varying temperatures. Experimental DMA data (solid line) and fit to US model (dotted line).

quoted growth rates of ca. 10^{-11} and 10^{-10} ms^{-1}, respectively) while Andronis et al. (1997) showed crystallisation isotherms at 30 °C at multiple humidities (and plotted rate constants varying from ca. 0.06–0.7 day^{-1} (4.12×10^{-5}–4.9×10^{-4} min^{-1}) as a function of water content). The literature data show the importance of water content on the rate of crystallisation (and so the importance of the water-permeable metal weave to hold the sample in the DMA).

The correction factor, r, is almost 1, which means that the model essentially reduces to that of Avrami and so the power term, n_{us}, becomes an indicator of the mechanism of crystal growth. Dealing with the 60 °C data first, at 60% RH or lower the index value is 2; since the glasses were cast from moulds and had very sharp edges, edge nucleation and growth seems a very reasonable mechanism. The index increases to 3 at 75% RH, representing a change in mechanism to growth in three dimensions, presumably because of the increased amount of water and plasticisation of the sample. Three dimensional bulk crystallisation to the α-polymorph was seen by Andronis et al. (1997) for indomethacin glasses held above 57% RH. At the lower temperature of 50 °C a mixed mechanism is indicated between surface and edge nucleation and growth. Andronis et al. (1997) noted surface nucleation for indomethacin glasses stored between 0 and 7% RH with a switch to predominantly bulk nucleation with storage above 21% RH, although their samples were held isothermally at a lower temperature (30 °C).

Fig. 10 shows the DMA data for a film of NaPHB held at 60 °C, 75% RH. Encouragingly there was an increase in the storage modulus value with time, as noted for the indomethacin glasses, and crystals of drug were seen in the film upon its removal from the instrument, indicating that crystallisation of the active had occurred. However, in order to effect the conversion to a crystallisation isotherm the initial value of the storage modulus is needed; it is apparent from the data in Fig. 10 that crystallisation started prior to measurement in the DMA and so quantitative analysis was not possible. This was perhaps not surprising given the fact that phenobarbital has a very rapid onset of crystallisation from the glass at 60 °C (0.29 h, Caron et al., 2009), which reduces slightly in the presence of a polymeric excipient (7 h with PVP, Caron et al., 2009). Nevertheless, the fact that changes in the storage modulus were seen with isothermal measurement of oral films by DMA gives encouragement that the method has potential as a stability-indicating assay for what are extremely difficult systems to study.

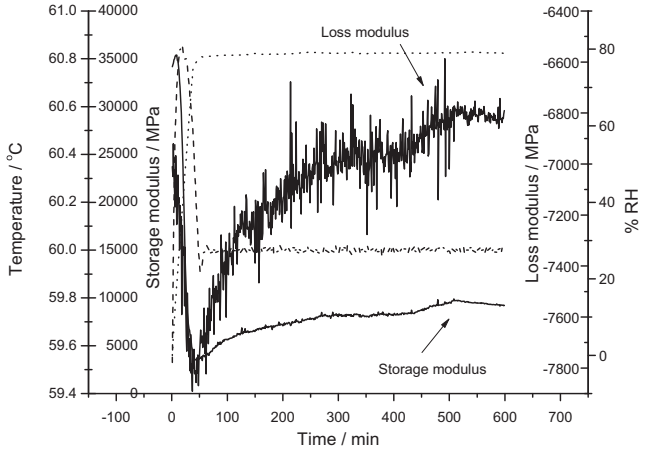

Fig. 10. DMA data for crystallisation of NaPHB film held at 60 °C and 75% RH.

4. Summary

A method for studying crystallisation of amorphous pharmaceuticals has been developed and tested. Sample pockets made of a metal mesh allow glasses to be mounted in the DMA and the Dutch twill weave pattern allows exposure of the sample to humidity. Crystallisation was manifest as an increase in the storage modulus signal with time. Conversion of the data to fraction crystallisation allowed quantitative determination of the rate and mechanism of crystallisation by application of the Urbanovici–Segal model. Rates of crystallisation were seen to increase with temperature and humidity. Temperature and humidity affected the mechanism of crystallisation. High temperature and humidity resulted in three dimensional crystal growth. Reducing the humidity caused a switch in mechanism to growth from edges. Reducing temperature resulted in a mixed mechanism of growth from surfaces and edges. The DMA was also sensitive to crystallisation of NaPHB formulated in an oral film, but quantitative analysis was not possible as the onset of crystallisation was not recorded. DMA offers potential as a stability-indicating assay for amorphous pharmaceuticals.

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