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The crystallisation of a model hydrophobic drug (terfenadine) following exposure to humidity and organic vapours

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Abstract

Terfenadine was selected as a model drug as it readily converts to the amorphous form, is chemically stable and is hydrophobic. Amorphous terfenadine beads were exposed to water vapour (different RH values) and the change in crystallinity was monitored using isothermal microcalorimetry. It was found that a small amount of the surface crystallised and then the process stopped (after about 9 weeks even though storage continued for ca. 2 years). The self-limiting crystallisation was due to water having limited access to the surface, believed to be at certain cracks and pores. Water sorption data were modelled such that it was possible to compare rates of sorption at different RH values and on fresh and aged material. It was shown that sorption was slower with reducing RH and also much slower on aged samples that had relaxed and reduced the size of surface defects. Mixtures of ethanol and water yielded a vapour that produced increasing rates of crystallisation as the ethanol content increased, but which only crystallised the outer surface of the beads and not the core. A mixture of ethanol and *n*-propanol caused complete crystallisation, because the propanol was sufficiently hydrophobic to absorb throughout the bead and lower Tg. An isothermal microcalorimetry method was described that allowed quantification of the amorphous content. The work has demonstrated the complexity of the crystallisation of hydrophobic amorphous materials when exposed to various vapours.

Keywords: Amorphous; Hydrophobic; Isothermal microcalorimetry; Gravimetric vapour sorption; Rate of sorption

1. Introduction

The amorphous form of drugs will have different physico-chemical properties to crystalline forms. On occasions the amorphous form is created intentionally, for example to improve dissolution rate, and often the amorphous form is created unintentionally during energetic processing, such as milling (Briggner et al., 1994). In either case it is essential to understand the properties of the amorphous form, especially with respect to its physical stability.

There are a great many publications relating to hydrophilic materials, showing how they can be studied following crystallisation in an isothermal

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microcalorimeter (as originally described by Briggner et al., 1994, and then recently by Otsuka et al., 2002; Mackin et al., 2002). Equally the use of gravimetric water sorption techniques as a means of detecting crystalline content, through sample crystallisation in the presence of humidity, (first described for the Dynamic Vapour Sorption balance by Buckton and Darcy, 1995) is now wide spread. This approach uses the loss of mass during a sorption cycle as being indicative of a crystallisation. Indeed it is now the case that workers erroneously interpret the absence of a mass loss in a water sorption experiment as being proof of complete crystallinity. There are two reasons why a (partially) amorphous sample has an absence of a crystallisation response, the first being that all the sorbed water can be used as a hydrate and thus there is no desorption (Hogan and Buckton, 2001), the second reason being that the sample is simply too hydrophobic to be crystallised by water absorption. There are comprehensive studies on the crystallisation of amorphous hydrophobic materials in the literature. For example Andronis et al. (1997) have shown gradual crystallisation of indomethacin following its storage at various temperatures and relative humidities. However, since the first study on the use of organic vapours in an isothermal microcalorimeter to induce crystallisation of a hydrophobic material (Ahmed et al., 1996), there have been comparatively few studies developing the use of isothermal microcalorimetry (IM) and gravimetric water sorption (GVS) for use with hydrophobic materials.

In this work the crystallisation of a model hydrophobic drug (terfenadine) was studied using IM and GVS in the presence of humidity and organic vapours. Terfenadine was selected as a model drug, despite the fact that its clinical use has declined, as it is hydrophobic and is easy to form into the amorphous state without degradation (Badwan et al., 1990). The aim of the work was to get a greater understanding of how to study the crystallisation of amorphous hydrophobic materials using IM and GVS, especially with respect to the ability of different vapours to diffuse into the amorphous form and the consequence of that diffusion. Both IM and GVS can be used to study the behaviour of samples in real time, often under equilibrium conditions (at least the environment in which the sample is stored can be at equilibrium). This is a good way of studying the effect of vapours on the crystallisation of samples. This cannot be done in either modulated or conventional differential scanning calorimetry (DSC), or thermogravimetric analysis, as the change in temperature with these techniques inevitably disturbs the distribution of the vapour in the sample.

2. Materials and methods

Amorphous terfenadine was prepared by melting crystalline terfenadine (Ricorolati, Italy, a gift from the Jordan Pharmaceutical Manufacturing Company) to 160 °C, then taking the molten mass and dropping it from a Pasteur pipette onto aluminium foil and leaving the drops to cool. The resulting beads were found to be amorphous, by absence of a melting peak (see Section 2.3). The amorphous beads had a diameter of ca. 3 mm and a mean weight of 11.7 mg (±0.72). The glass transition temperature of the amorphous beads was 58.5 ± 1.3 °C, with the absence of a melting peak when tested on the DSC (see method below). The original crystalline raw material of terfenadine has a peak melting point of 152.4 ± 0.6 °C and an enthalpy of fusion of 101.5 ± 0.8 J/g. Badwan et al. (1990) reported that the most stable polymorph of terfenadine has a peak melting point of 151.1 °C and an enthalpy of fusion of 102.0 J/g.

2.1. Isothermal microcalorimetry (IM)

Experiments were carried out by adding 10 amorphous beads to a 3 ml glass ampoule. A Durham tube containing either a saturated salt solution (KCl providing a relative humidity of 85% at 25 °C), or a mixture of ethanol and water (range 25–60%, v/v ethanol) or ethanol and *n*-propanol (50%, v/v ethanol *n*-propanol) was added and the ampoule was sealed with a rubber stopper and aluminium over seal. The ampoules were loaded into the measuring site of an isothermal microcalorimeter (Thermal Activity Monitor, Thermometric), which was operated at 25 °C. In all cases the reference side of the calorimeter was loaded with a freshly sealed ampoule containing a Durham tube with the same fluid, but without the amorphous terfenadine.

2.2. Gravimetric vapour sorption (GVS)

Six beads of amorphous terfenadine were placed on the glass measuring pan of a Dynamic Vapour Sorption

apparatus (Surface Measurement Systems, UK). Experiments were performed at 25 °C and involved exposing the sample to a selected RH for 20 h.

2.3. Other methods

Differential scanning calorimetry (DSC) was carried out using a Perkin–Elmer DSC 7, using non-hermetically sealed aluminium pans and a scan rate of 10 °C/min, under nitrogen purge, having used indium to calibrate the system.

Scanning electron microscopy (SEM) was performed using a Phillips XL20 instrument following sputter coating using an Emitech K550 for 3 min at 40 mA. X-ray diffraction was measured using a Phillips PW 1840 diffractometer, scan range 2–30° 2θ, receiving slit width 0.2 mm.

2.4. Storage experiment

IM was used to assess the crystalline content of samples that had been stored for different times at either 44, 75 or 86% RH at 21 ±1 °C. Ten beads of amorphous terfenadine were loaded into each IM ampoule, with each ampoule being left open in desiccators containing saturated potassium carbonate (44% RH), sodium chloride (75% RH) or potassium chloride (86% RH). At each test point an ampoule was removed from the desiccator, a Durham tube containing 50:50 ethanol:*n*-propanol was added and it was sealed and immediately tested in the calorimeter. Different ampoules were removed from the desiccator at each time point. Three ampoules were tested for each time point and the mean value reported.

3. Results and discussion

3.1. Isothermal microcalorimetry

All isothermal microcalorimetry experiments at 25 °C produced flat baseline responses (after the initial disruption due to the lowering of the ampoule into the instrument) over a 24 h period. As it was known that the sample was amorphous, it is clear that the absence of a peak in IM experiments at high RH demonstrates the absence of a measurable crystallisation response rather than the presence of a crystalline sample.

The use of mixtures of ethanol and water in the IM gave rise to crystallisation events if the ethanol content was 40% (or greater), but no response was seen if the ethanol content was 25% (Fig. 1). It is clear from Fig. 1 that the area under the curve is largest for the 60:40 ethanol:water mixture and gets progressively smaller as the ethanol content decreases (values of AUC were 47.1, 26.6 and 14.6 J/g for 60, 50 and 40% ethanol in water, respectively). When the samples were removed from the IM they were tested by DSC and all were found to show a crystallisation response (a wide exotherm at around 100 °C, figures not shown). A wide exotherm was seen indicating a gradual yet complete crystallisation (as the enthalpy of fusion matched that of the crystalline sample) of the amorphous regions remaining in the sample. The original amorphous beads did not crystallise in the DSC at the same heating conditions and only a Tg was seen. This can be explained as being due to an easier crystallisation in the presence of seeding

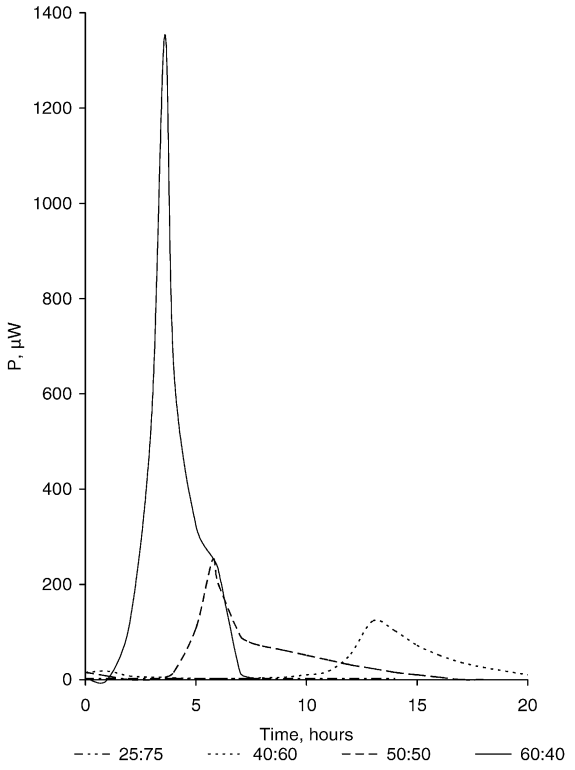

Fig. 1. Microcalorimetric responses for amorphous terfenadine exposed to the vapour of 25, 40, 50 and 60% ethanol in water mixtures.

regions and some plasticizing effect of the sorbed vapour. The measured Tg after exposure to 40:60 ethanol:water was 56.2 °C and after exposure to 50:50 ethanol:water it was 55.0 °C It was clear therefore that exposure to ethanol:water mixtures caused only a partial crystallisation of the amorphous sample, even though the onset was rapid for the high ethanol content vapour. A bead was removed from the IM and sliced with a scalpel and then studied using scanning electron microscopy (Fig. 2). It was clear that the exposure to ethanol/water mixtures had caused the outer surface to crystallise, whilst the core was still amorphous. To date there have been no reports of rapid and large crystallisation responses for samples in which not all of the material has crystallised. It follows that the response for hydrophobic samples is complicated, but that ethanol can plasticize the surface, but the surface crystallisation seems to limit further sorption and prevent complete crystallisation of the sample. It is extremely unlikely that the vapour is uniformly distributed throughout the sample, but rather more likely that it is preferentially at the sample surface. DSC is not a good tool for the study of distribution of vapour as the vapour will move on heating, hence the measured Tg for such a complex system is difficult to interpret. It is likely that for the crystallising sample the Tg is lower at the surface (high vapour content) and higher in the bulk (limited vapour content).

IM experiments were adapted using mixtures of ethanol and *n*-propanol in order to find a mixture that resulted in complete crystallisation of the amorphous sample and to have the entire response captured by the calorimeter (i.e. not to have so fast an onset that part of the response was lost during the loading and temperature equilibration of the calorimeter cell). It was found that a 50:50 mixture of ethanol and *n*-propanol was a suitable mix to use. The sample removed from the IM following exposure to this mix was found to have no crystallisation response by DSC and to yield an enthalpy of fusion identical to that of the original sample (102 J/g, the sample having crystallised to the same polymorphic form as the original – checked by X-ray diffraction and the melting point and enthalpy of fusion). The IM response with this mixture of ethanol and *n*-propanol was a large peak with several shoulders, indicating that the crystallisation progressed in discreet leaps through the sample, presumably as the vapour saturated regions further into the bulk of the bead. The presence of shoulders following the main sharp peak in the IM response was reproducible and can be explained as being due to a gradual crystallisation. Ahmed et al. (1996) had a similar finding and they argued that the presence of shoulders or shallow peaks subsequent to the initial crystallisation event is a result of a more gradual crystallisation superimposed with expulsion of ethanol vapour (endothermic) and condensation of ethanol back into solution (exothermic).

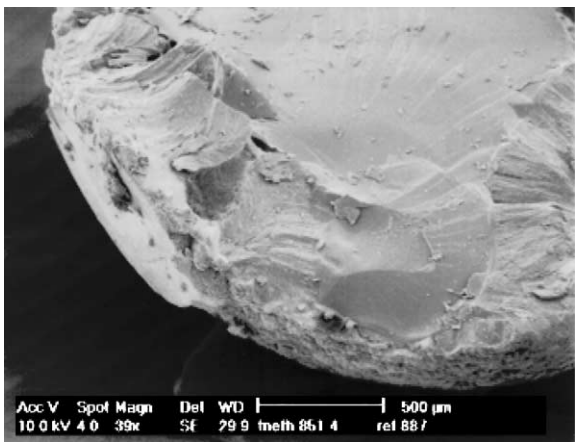

Fig. 2. Sliced bead, showing amorphous core and crystalline surface, following exposure to ethanol/water vapour in the IM.

3.2. Using IM to quantify amorphous content after storage at different RH

Samples of amorphous terfenadine were stored at 44, 75 and 86% RH and removed at different times (21 °C). These samples were then exposed to a 50:50 ethanol:*n*-propanol mixture in the IM to cause the crystallistaion of any remaining amorphous content. The amorphous content remaining was quantified by the ratio of the area under the curve for crystallisation for the test sample and a fresh amorphous sample. The data for the storage study are shown in Fig. 3. It can be seen (Fig. 3) that the material starts to crystallise relatively quickly, but that after 3 weeks the sample stored at 44% RH shows no further crystallisation and after 9 weeks the samples stored at both 75 and 86% stop crystallising. Scanning electron micrographs showed that the samples had partially crystallised surfaces, but still had substantial amorphous content apparently

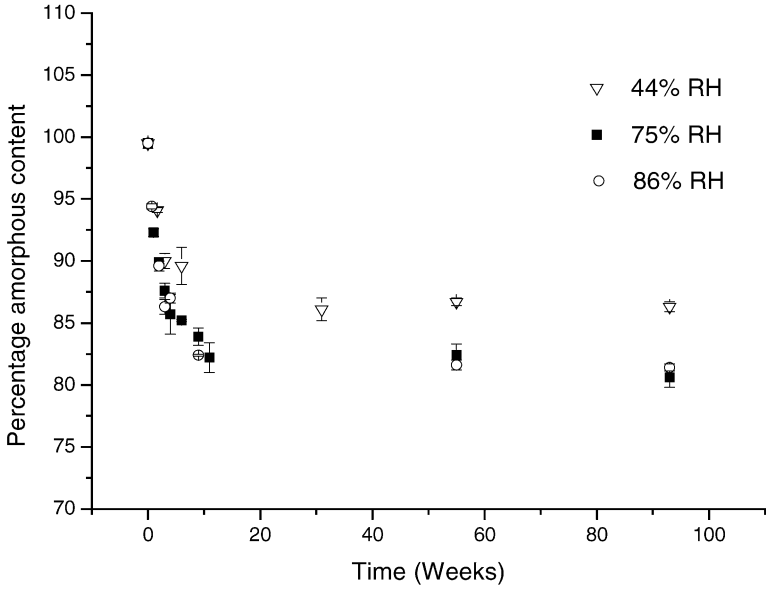

Fig. 3. Percentage amorphous content remaining following storage of samples at 44, 75 and 86% RH for 93 weeks.

accessible at the surface (Fig. 4). A levelling off of crystallisation has been reported previously for amorphous samples due to a decreased strength of solid–water interactions resulting in reduced plasticizing efficiency (e.g. Slade and Levine, 1991; Hancock and Zografi, 1993). However, it is surprising that the sample here should show such a dramatic change in rate and sudden stop in crystallisation (Fig. 3) when the surface of the particles are far from completely crystalline (Fig. 4a). It appears (Fig. 4b) that all the surface crystallisation regions are started at surface defects (either cracks of large pores), but equally that not all large pores act as a centre for crystallisation (in Fig. 4a it can be seen that there are substantial defects on the surface in the amorphous region). Thus to conclude, it must be true that water vapour at RH up to 86% and at 21 °C, is not able to penetrate the surface of the amorphous terfenadine beads enough to result in plasticization and thus there is no rapid crystallisation. However, there clearly are some surface sites, associated with cracks and pores, where it is possible for water to gain access and start a process of crystallisation. Rather than spreading continuously across the surface (or into the bulk), these regions become self limiting, which must be viewed as having the crystalline regions sealing the areas on the surface of the beads to which water was once able to absorb. Whether these regions are initially accessible due to geometry or due to the exposure of less hydrophobic functional groups of the terfenadine molecule is not clear, but it is true that not all defects act as a site for surface nucleation. These observations were not expected and we believe they provide new information about the crystallisation of hydrophobic materials. It is distinctly possible that the crystallisation by the mixture of ethanol:*n*-propanol vapours starts at surface defects, but then progresses to complete crystallisation, as all the crystallised particles have a common shape, possibly due to shrinkage from a pore of onset as the amorphous material packs into the crystalline form (Fig. 4c).

In order to try to understand the factors that govern the crystallisation of the amorphous drug, water sorption data were collected by exposing the sample to 44, 75 and 90% RH. Sorption was seen to continue for 20 h with no sign of reaching a plateau (e.g. Fig. 5). The data (at all three RH values) had a very good fit (see Fig. 5 as a typical example of the quality of fit) to the expression:

$$\frac{100(M - M_0)}{M_0} = k(t - t_0)^{0.57} \quad (1)$$

where M is the mass of the sample at time t, M_0 the initial mass of sample at time t_0, and k the sorption rate constant. The exponent 0.57 provided the best fit to the

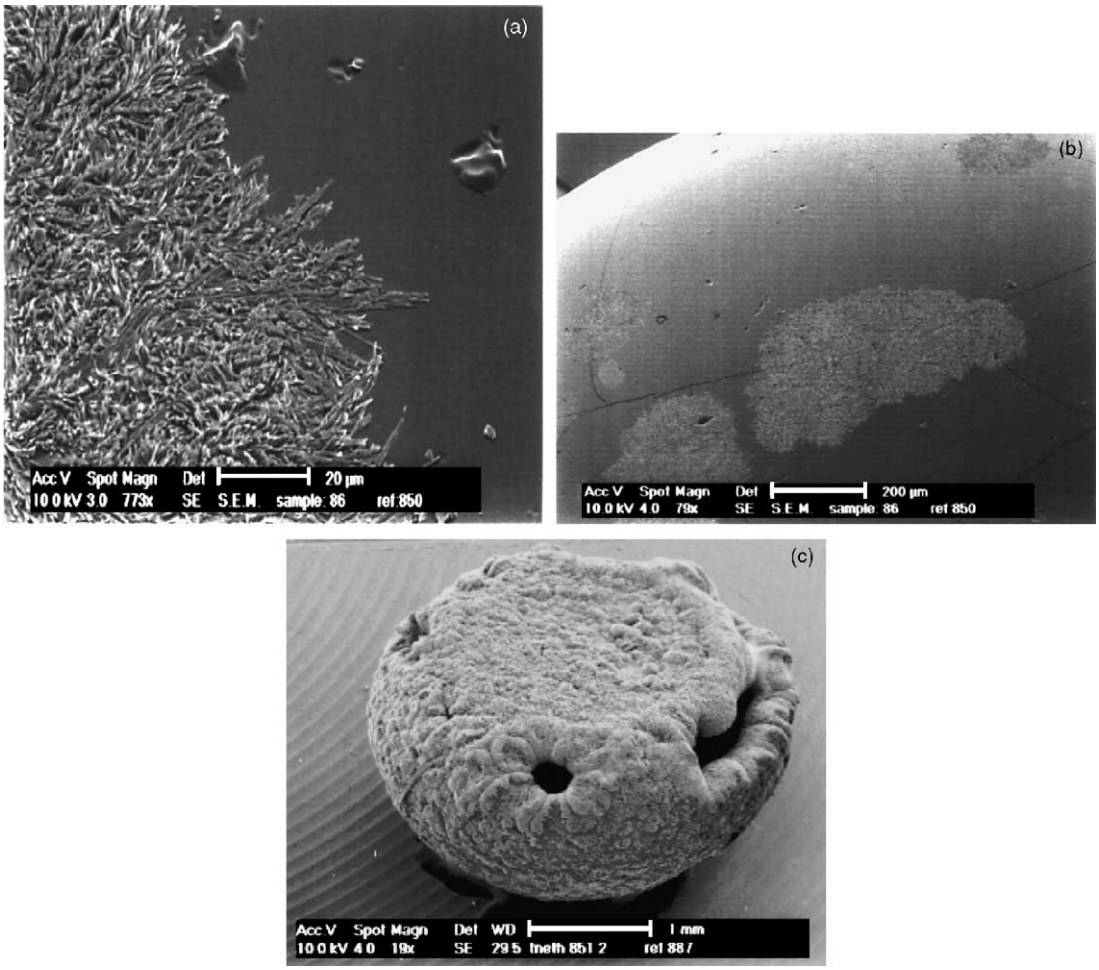

Fig. 4. (a) SEM of partial surface crystallisation of an amorphous terfenadine bead, following storage at 86% RH for 1 year. (b) Crystallisation of amorphous terfenadine following storage for 1 year at 86% RH, showing regions of crystallisation associated with surface defects. (c) A fully crystallised particle, having been exposed to ethanol:*n*-propanol vapour, showing large pores which may have grown from access points for vapour.

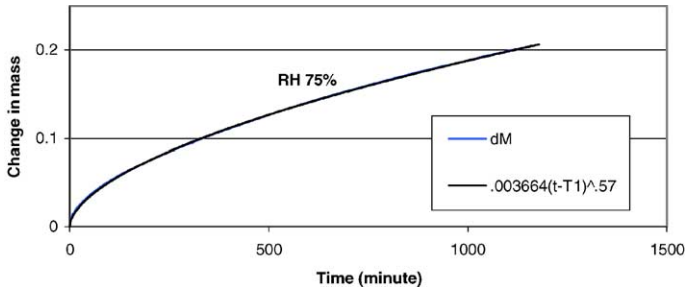

Fig. 5. Typical moisture sorption for fresh amorphous terfenadine at 75% RH, with superimposed curve fitting data for Eq. (1).

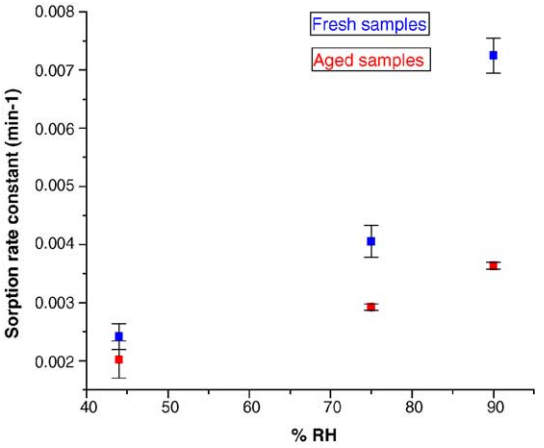

Fig. 6. Sorption rate constant as a function of RH for freshly prepared and aged (stored at 0% RH for 1 year) amorphous terfenadine.

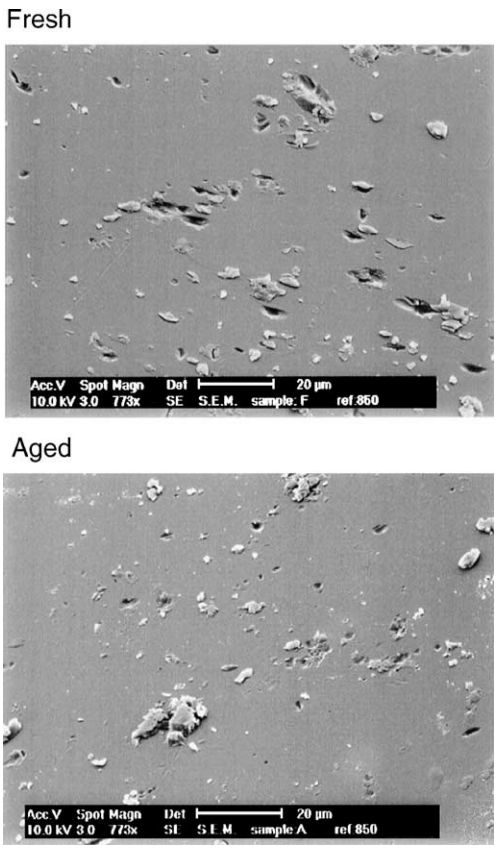

Fig. 7. Comparison of SEM images of fresh and aged particles, showing smaller pores on the aged particles.

water sorption data. Using this approach it was possible to compare the rate of water sorption at different RH values and also for fresh and aged samples. The values for the rate constants are shown in Fig. 6 for freshly prepared and aged samples of amorphous drug. The aged samples had been stored at 0% RH for 1 year. SEM pictures (Fig. 7) showed that the aged samples had much smaller surface defects, with pores being very much smaller and much less deep than those seen on SEM pictures of fresh materials, which is indicative of the sample having relaxed during storage. The fact that the rate, and presumably extent, of water sorption is much slower on samples with smaller pores adds weight to the hypothesis that crystallisation is started by preferential water sorption to some of the pores on the surface.

4. Conclusion

Hydrophobic drugs do not crystallise readily when exposed to water vapour. The data presented here show that water vapour gained access to certain defects on the surface or amorphous beads, but that the crystallisation response in these regions was self-limiting and caused further water access, and thus crystallisation to stop. The water diffusion into amorphous beads was modelled from DVS data to allow calculation of rate constants. The rate was, as expected, lower for low RH exposure. Aged material was found to have relaxed in a way that slowed the rate of sorption of water into the beads.

Ethanol vapour was able to access more of the particle (as evidenced by the higher extent of crystallisation), such that all of the surface crystallised, but then the vapour was not able to penetrate into the bulk of the particle so crystallisation again stopped. The extent of crystallisation that occurred could be altered by preparing mixtures with different ethanol:water composition (with the crystallisation decreasing with decreasing ethanol content). The mixture of ethanol:*n*-propanol 50:50 was found to cause complete crystallisation (indicated by the fact that the crystallised samples had an enthalpy of fusion in keeping with original crystalline material (102.5 ± 1.8 J/g, $n = 16$)) and to be detectable using isothermal microcalorimetry. This was a suitable method to estimate the amorphous content remaining in the stored beads.

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