Environmental Microscopy for the Pharmaceutical Sciences

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INTRODUCTION
The influence of environmental fluctuations on the stability of pharmaceutical solids is of great importance to the formulation scientist. Relatively minor changes in humidity can have major implications on the performance of a formulation [1-3]. For example, moisture present in the vapour phase can induce phase transitions such as dehydration [4], crystallisation [5] and deliquescence [6] in a pharmaceutical powder. Such transitions would almost inevitably lead to an unpredictable change in formulation performance.

Furthermore, globalisation and mass travel could potentially expose commonly used prescription medicines to large variations in climate (for example, 0% RH in north Africa to 100% RH in S.E. Asia [7]). Such implications highlight the need for experimental observation of formulation components under specific temperature and humidity conditions. Although previous investigations [8] have demonstrated optical microscopy to be a capable tool for identifying such changes in pharmaceutical solids, the control of humidity has usually been static (controlled by saturated salt solutions) with limited control of parameters during sample observation.

Here we apply a novel environmental control microscope stage to observe physical transformation as a function of humidity of a commonly used pharmaceutical drug.

MATERIALS AND METHODS
Salbutamol sulphate, commonly used for inhalation therapy (e.g. Ventolin) was spray dried using a mini-spray dryer (Büchi BL91, Flawil, Switzerland) to produce amorphous material (confirmed by X-ray powder diffraction).

The influence of humidity on the recrystallisation of the amorphous salbutamol was investigated using a novel environmental control stage for optical microscopy (VGI 2000M, Surface Measurement Systems Ltd, London, UK). Briefly, the VGI stage operates by combining a dry and humidified gas flow (using two independent mass flow controllers) in a sealed microscope stage. In addition, constant temperature of the stage was maintained by a Peltier-controlled water bath to eliminate condensation. Relative humidity could be controlled by varying the dry to wet flow ratios (mass flow values) and monitored by a built-in thermometer and humidity meter. The whole system was computer controlled, allowing both static and gradient measurements. A schematic diagram of the equipment set-up and VGI microscope stage are shown in Figs 1 and 2, respectively. The VGI humidity probe and mass flow controllers were calibrated against a sodium chloride standard, which deliquesces at 75.3% RH at 25°C.

A small quantity of the amorphous salbutamol sulphate was quickly transferred to a glass slide, mounted on the VGI stage, sealed and exposed to 0% RH for 4 hours. The VGI stage was positioned under a conventional Nikon transmission light microscope with a polarised-light filter, and imaged at a 160X magnification using a 160X magnification using a CCD camera attachment. The salbutamol sulphate was exposed to a humidity range of 0-90% RH at 10% RH increments. A pause of 100 minutes was insti-
to 10% RH increments over a period of 900 minutes.

Selected images from the amorphous salbutamol sulphate exposed to a 10% RH increment humidity ramp are shown in Fig 3. No discernible change in sample morphology was seen as the sample was exposed to 0, 10, 20, 30 and 40% RH for 100-minute periods, suggesting that the amorphous material was relatively stable across this range. Exposure to 50% RH for 100 minutes resulted in sample darkening. This is most likely due to densification of the amorphous material as the amount of water in the sample approaches the critical value required to lower the T_g to 25°C. Exposure to 60% RH for 100 minutes resulted in complete structural collapse of the salbutamol sulphate, indicating that the molecular mobility was sufficient to allow reorientation and potential crystallisation. Furthermore, these observations would suggest the critical RH (for a T_g of 25°C) to be between 50 and 60% RH, corroborating observations made by previous investigators [9].

However at such a humidity, crystallisation of salbutamol sulphate will be very slow (in the order of days). Exposure to 70% RH was sufficient to allow a rapid crystallisation of the collapsed amorphous salbutamol, and can be seen in Fig 3 under polarised light. As expected, further increments in humidity (80 and 90% RH) resulted in no visible change in morphology. Although previous investigations [5] have suggested such crystallisation to be a long process, kinetics will be related to sample size and therefore will be quicker than that obtained using bulk methods such as dynamic vapour sorption.

At increased humidities, the molecular mobility in the collapsed material will be higher, resulting in increased crystallisation kinetics. This was demonstrated by exposing the 0% RH equilibrated amorphous salbutamol sulphate to a static humidity of 80% RH. Selected images taken at 4 minute intervals at 80% RH are shown in Fig 4. Again, at 0 minutes the amorphous salbutamol sulphate appears as solid particulates (with no observable change with respect to time). Upon exposure to 80% RH, a rapid collapse in the structure was observed as the amorphous material absorbed water and molecular mobility increased. After 4 minutes, many of the distinct particulates, observed at 0% RH, become part of supersaturated liquid. Many of the individual droplets, relating to the solid particulates at 0% RH, coalesce into the large mass due to surface tensions, until at 12 minutes the collapsed material began to crystallise.

At 16 minutes the crystallisation became obvious as the formation of long-range crystal structure was observed under polarised light. After 20 minutes, no change in the morphology, or degree of polarised light could be observed, suggesting that the crystallisation of amorphous salbutamol sulphate was complete.

**RESULTS AND DISCUSSION**

It is well documented that the sorption of moisture from the vapour phase into amorphous material enables the randomly orientated molecules throughout the structure to reassemble into a more thermodynamically stable crystal form. Such long-range order can only be achieved when the molecular mobility is increased sufficiently to lower the materials glass transition temperature (T_g) to that of the environment. Thus, a critical RH will exist at which an amorphous material will recrystallise. In order to demonstrate such phenomena the amorphous salbutamol sulphate was exposed

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