Stored amorphous samples and intrinsic dissolution testing – the interplay of crystallisation and dissolution behaviour

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The recrystallisation behaviour of amorphous indomethacin and the dissolution behaviour of different solid state forms of indomethacin have been previously studied. However, the effect of crystallites developed during storage on recrystallisation during dissolution has not yet, to the best of our knowledge, been investigated.

Quench cooled amorphous indomethacin stored at 30 °C and 23% or 42% relative humidity (RH) was characterised by dissolution testing. Crystallisation during storage and dissolution testing was monitored with Fourier transform attenuated total reflection infrared (FT-ATR-IR) spectroscopy and scanning electron microscopy. Freshly prepared indomethacin recrystallised to the α-form during dissolution. Indomethacin particles stored at 23% RH exhibited surface crystallisation (γ form) during storage (5 days), recrystallised to the γ form during dissolution and exhibited a dissolution profile similar to the γ polymorph. Indomethacin stored at 42% RH recrystallised to the γ form. After storage (5 days), the tablet surface crystallised during dissolution to an α-γ-mixture according to FT-ATR-IR spectroscopy. After storage for 14 days, dissolution resulted in recrystallisation mostly to the γ form. This work suggests that crystallite formation in amorphous systems during storage under different conditions influences the crystallisation behaviour of the remaining amorphous material during dissolution testing.

Keywords: Recrystallisation, dissolution, amorphous, indomethacin, FT-ATR-IR spectroscopy

Introduction

The most important advantage of amorphous material from a pharmaceutical perspective is the higher solubility of the amorphous form compared to that of the respective crystalline material. Therefore amorphous drugs dissolve faster and to a higher extent than their crystalline counterparts [1, 2]. However, amorphous material is thermodynamically unstable and will eventually recrystallise to the energetically preferred crystalline form either during storage, during dissolution or both [3-5].

The recrystallisation of amorphous drugs has been studied with emphasis on various aspects such as storage conditions [6, 7], resulting solid state forms (different polymorphs or hydrates) [6], kinetics of the recrystallization process [8, 9], and distribution of the resulting crystallites [10].

The crystallites (or seeds) formed during storage should have an effect on the resulting polymorph and the dissolution profile. In this study, the effect of crystallites, formed after storing amorphous material for different storage times and at different humidities, on the dissolution behaviour and crystallisation behaviour during dissolution testing has been investigated.

Materials and Methods

Indomethacin (IMC) was obtained from Hangzhou Dayangchem, China. The analytical grade methanol for preparing α-IMC was purchased from Merck, Darmstadt, Germany. Potassium dihydrogen phosphate and potassium acetate were purchased from Scharlab S. L., Mas d`En Cisa, Spain. Tween 20 and sodium hydroxid were obtained from Sigma Aldrich Chemie, St. Louis, USA and from BDH, Poole, England respectively.

The γ form of IMC was heated to 167 °C, quench cooled with liquid nitrogen, equilibrated at 25 °C for 30 min, ground and sieved and the size fraction of 150 -250 µm was used for this study. The amorphous samples were stored over super saturated salt solutions of potassium acetate (23% RH) and potassium carbonate (42% RH) at 30°C.

Fourier transform attenuated total reflection infrared (FT-ATR-IR) spectroscopy measurements were conducted on a GladiTech Varian 3100 Excalibur Series FT-IR spectrometer (Varian Incorporated, California, USA) with a KBr beamsplitter and a cooled DTGS detector. The spectrometer was equipped with a GladiATR^{TM} accessory for FTIR spectrometers (PIKE technologies, Madison, USA) using a monolithic diamond as ATR crystal. A background scan was taken and automatically subtracted from subsequent measurements.

Intrinsic dissolution studies were performed on compacts (150 ± 2 mg of sample) using an Erweka DT600 rotating disk dissolution apparatus (Heusenstamm, Germany). The dissolution studies were performed in 900 ml of dissolution medium (simulated intestinal fluid without enzymes at a pH of 6.8 ± 0.05 with 0.05% Tween 20) per vessel, the temperature was held at 37 ± 0.5 °C and disks were

rotated with a speed of 50 rpm. Samples (5 ml) were taken at predetermined time points (5, 10, 20, 30, 45 and 60 min) and the concentration of dissolved drug was quantified at a wavelength of 318 nm with UV/Vis spectroscopy. After the dissolution experiment, FT-ATR-IR measurements were performed on the undissolved compact surfaces.

For scanning electron microscopy (SEM) analysis, stored particles and compacts after dissolution were cut with a razor blade. The upwards facing cross sections of particles or compacts were fixed on double sided carbon tape and sputter coated with gold palladium. A field emission scanning electron microscope (JEOL Ltd, Tokyo, Japan) fitted with JEOL 2300F EDS system (JEOL Ltd, Tokyo, Japan) with a 15 kV beam acceleration voltage was used for SEM imaging. All samples were prepared and analysed in triplicate.

Results and Discussion

Amorphous samples - storage

Amorphous indomethacin stored at 23 and 42% RH recrystallised to the γ polymorph; however, samples differed with regards to the crystallite location. When stored at low humidity, crystallisation started at the particle surface, however, when stored at medium humidity crystallites could be seen also within the particles (Figure 1).

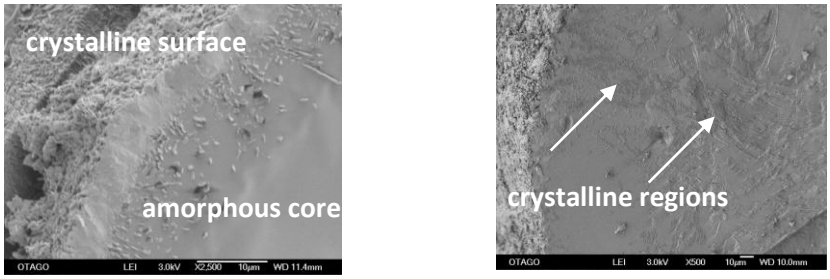

Figure 1: SEM images of cross sections of IMC particles stored for 5 days at 23% RH (left side) and stored for 42 days 42% RH (right side).

Stored samples – dissolution

To investigate the influence of the crystallite amount and location on dissolution behaviour and crystallisation behaviour during dissolution testing, intrinsic dissolution studies were performed on freshly prepared amorphous IMC and samples stored at 23 and 42% RH at day 5 and day 14. The dissolution behaviour was recorded and the surface of the compact after the dissolution testing was probed with FT-ATR-IR spectroscopy. The data was analysed using principal component analysis (PCA). Freshly prepared amorphous indomethacin showed a significantly higher dissolution rate than the crystalline forms (Figure 2), however, the compact surface recrystallised to the α polymorph during dissolution (Figure 3).

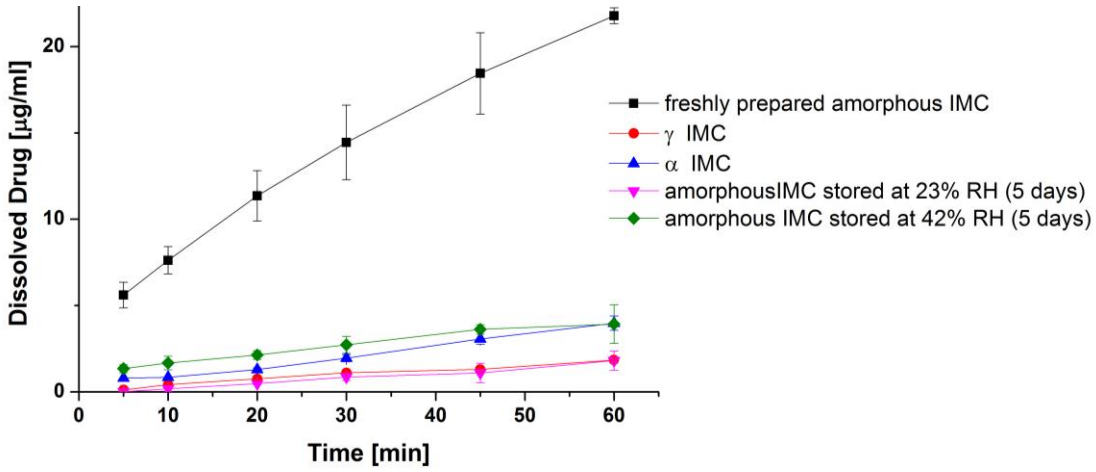

Figure 2: Dissolution profiles of freshly prepared amorphous, α-and γ- IMC, amorphous IMC stored at 23% RH (5 days) and amorphous IMC stored at 42% RH (5 days), n = 3, mean ± SD.

Samples stored at 23% RH resulted in a dissolution profile similar to the γ form and in the PCA of the FT-ATR-IR measurements stored samples clustered with the γ form (Figure 3). This was probably due to the crystallites being located on the particle surface (as identified with FT-ATR-IR) acting as seeds which led to crystallisation of the whole compact.

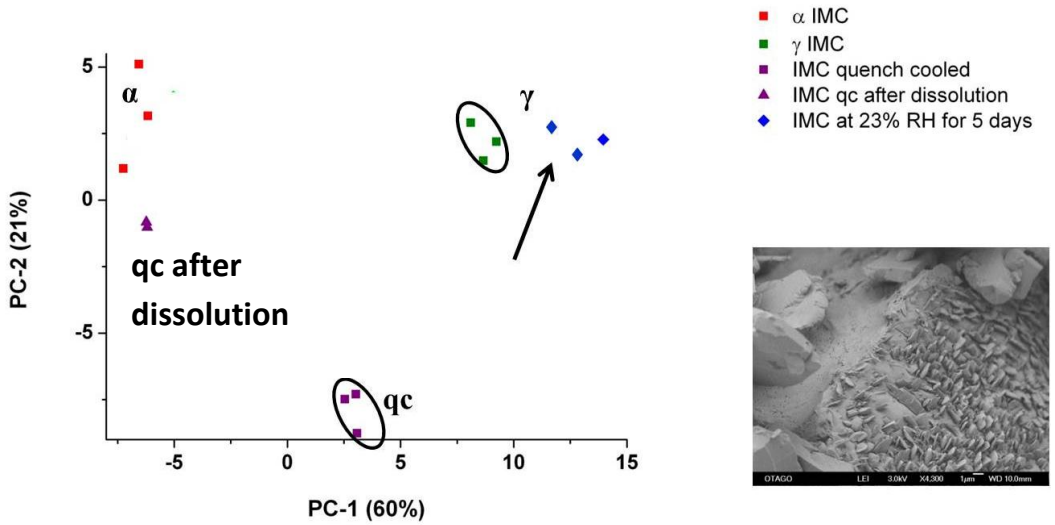

Figure 3: PCA scores plot of IR measurements of IMC (stored at 23% RH for 5 days) after dissolution and SEM cross section of the tablets after dissolution

Dissolution testing performed after 5 days of storage at 42% RH resulted in a dissolution profile similar to the α form, however, in the PCA scores plot these samples are located between the α and γ forms, but closer to the α form. This might be explained by crystallisation during dissolution resulting in the α form and the effect of crystallites present due to the storage resulting in the γ

form. Analysis of samples stored for 14 days and then subjected to dissolution testing, revealed the samples were more similar to the γ form in the PCA scores plot, indicating the increased effect of crystallites from storage (Figure 4).

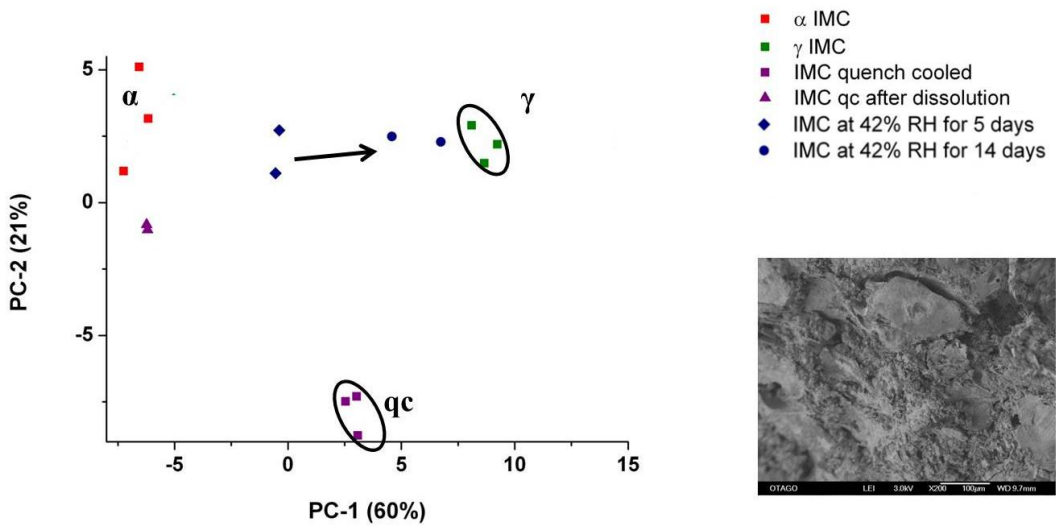

Figure 4: PCA scores plot of IR measurements of IMC (stored at 42% RH for 5 and 14 days) after dissolution and SEM cross section of the compact after dissolution(day 14).

Conclusions

Amorphous indomethacin recrystallised at different localisations within the particles depending on the humidity samples were stored at. This work suggests that crystallite formation (presence, distribution and amount) influences not only the dissolution behaviour, but also the crystallisation behaviour of the remaining amorphous material during dissolution testing, especially with regards to the resulting polymorph.

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