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Quantification of crystalline forms in active pharmaceutical ingredient and tablets by X-ray powder diffraction

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Abstract

A Merck development compound was known to exist in several polymorphic forms, hydrates and solvates. The polymorphic forms were characterized and the most thermodynamically stable form at room temperature was identified and taken into development. During routine stability analysis it became apparent that the crystalline form of the compound was converting from one form to another in tablets that were stored at 40 °C/75% relative humidity in open containers. This form conversion did not occur when the active pharmaceutical ingredient (API) alone was stored under these conditions. This paper describes the development and application of an X-ray powder diffraction method for the determination of the relative content of the two crystalline forms in API and within the final formulation. Results of monitoring the crystalline form conversion are reported and a possible mechanism of conversion is postulated.

Introduction

Polymorphism is of major interest to the pharmaceutical industry. It is well known that different polymorphs, hydrates and solvates can have different physicochemical properties such as solubility, melting point, dissolution rate, chemical stability, wettability, etc. (Haleblian & McCrone 1969; Haleblian 1975). Some of these properties can ultimately affect the bioavailability of a compound and hence the efficacy or toxicity of a pharmaceutical product. It is therefore wise to screen extensively for polymorphs, hydrates and solvates of a novel pharmaceutical compound and fully characterize those found in order to determine which is the most suitable form to take into development.

Several methods are available to monitor the physical forms of active pharmaceutical ingredients, either qualitatively or quantitatively. These methods include infrared spectroscopy (Agatonovic-Kustrin et al 1999), solid-state nuclear magnetic resonance spectroscopy (Saindon et al 1993; Gao 1996), near-infrared spectroscopy (Gimet & Luong 1987; Patel et al 2000), differential scanning calorimetry and thermogravimetric analysis (Giron & Goldbronn 1997), Raman spectroscopy (Findlay & Bugay 1998) and X-ray powder diffraction (XRPD) (Chao & Vail 1987; Giron et al 1990; Suryanarayanan & Herman 1991; Yamamura & Momose 2001). Only a few of these cited examples deal with formulated pharmaceutical products. Each of these methods will be affected to some extent by excipients that may completely obscure the areas of interest in the spectrum or diffractogram.

XRPD is generally considered the method of choice for qualitative determination. Different polymorphs, hydrates and solvates can usually be clearly identified by their powder X-ray patterns. Quantitative XRPD is a commonly used technique, especially for inorganic materials. The application of quantitative XRPD to organic materials of pharmaceutical interest, i.e. the active pharmaceutical ingredient (API) and formulations, is much less widespread and none of the cited examples shows an actual crystal form conversion occurring within a final formulation.

One of our development compounds had been shown to exist in several different polymorphic, hydrated and solvated forms. Form I is an anhydrous polymorph of the API and was chosen as the form to be used for development. The formulated product was

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placed in controlled environmental chambers for routine chemical stability testing. The physical form of the API was also monitored closely. After only a few days it became apparent that the form of the API in the tablets stored at 40 °C/75% relative humidity (RH) in open containers was changing from Form I to Form II. Form II is a tunnel hemi-hydrate form of the API. This paper describes the attempt to monitor and quantify the extent of this conversion using XRPD.

Materials and Methods

X-ray diffraction patterns were obtained using a Bruker D8 Advance X-ray powder diffractometer in the Bragg–Brentano (theta–theta) geometry with a 435 mm measuring circle. The X-ray source used copper radiation Kα1 and Kα2, with a tube power of 1600 W (40 kV and 40 mA). The detection was performed using a scintillation counter. Data from API mixtures were collected using a step size of 0.02° 2θ and a time-per-step of 1 s. Data from simulated tablet mixtures and tablets were collected using a step size of 0.01° 2θ and a time-per-step of 5 s. All sample data were collected using the variable anti-scatter and detector slit program set to give a 12 mm sample illumination. Samples were rotated at 60 rotations per minute to help reduce preferred orientation. The samples were prepared and analysed using sample holders containing silicon plates cut in the (5,1,0) plane to minimize background radiation. The plates contained a 1-mm deep by 12-mm diameter well cut into the silica to allow reproducible sample preparation of approximately 100 mg of material. Samples were prepared level, but with a slightly roughened surface to try to minimize preferred orientation effects.

Form I tablet preparation, storage and analysis: API and excipients were wet granulated and mixed to produce a granule that was then dried in a fluid-bed dryer. The dried granule was co-milled, followed by lubrication in a blender. The tablets were compressed on a single punch tablet press and finally film-coated. The tablets contained 25% API, 38% lactose monohydrate, 28% microcrystalline cellulose as well as pharmaceutically acceptable binder, disintegrant, lubricant and wetting agent. Tablets were stored in environmental chambers maintained to within ± 2 °C and ± 5% RH of claim in either open or sealed high density polyethylene (HDPE) bottles. Prior to analysis, individual tablets were removed and crushed in an agate pestle with a mortar, the pieces of film coat were removed and the remaining tablet material gently ground to a fine powder.

Known API mixtures of Form I and Form II were prepared by weighing and mixing samples of the pure forms. The samples were weighed on a Sartorius MC210S balance that has been regularly calibrated and monitored to ensure weights conform to at least 0.1% of claim. Simulated tablet mixes were prepared to contain 25 ± 1% API and 75 ± 1% of powdered placebo tablet.

Statistical analysis

Statistical analysis was performed on the calculated vs actual Form I content for the API and for the simulated tablet mixtures. Linear regression was performed on both sets of data. The experimental results were compared with the theoretical equation $y = mx + c$ where m is one and c is zero. Within 95% confidence intervals the gradient in each case was shown to be one and the intercept was zero. Using the 95% confidence intervals the expected error was 4–5% for measurements on the API and 6–7% for measurements on the simulated tablets.

Repeatability of measurement was determined by five repeat measurements of a simulated tablet containing approximately 50% Form II and an actual tablet containing approximately 40% Form II. Within 95% confidence intervals a variation in measured results of up to 7% in the simulated tablet and 1% in the actual tablet formulation would be expected.

Results and Discussion

XRPD crystal form quantification

In order to obtain a method for quantitative analysis by XRPD, the diffractograms of the two crystalline Forms I and II and of a placebo tablet were examined for distinct differences. From this comparison the two peaks of Form I at *c*. 7.5° 2θ and the peak of Form II at *c*. 8.0° 2θ were deemed the most likely to form the basis of an acceptable method. They are separated by sufficient distance to allow for quantification and are free from interference from peaks due to the excipients in the tablet. A comparison plot showing diffractograms of Form I, Form II and tablet placebo is shown in Figure 1.

Since polymorphs of a given compound possess identical mass absorption coefficients, the diffraction intensities due to a specific polymorph are directly proportional to the composition of that polymorph in a mixture (Chao & Vail 1987). In our case it was assumed that the presence of up to half a mole of water in the tunnel hydrate Form II would have a negligible impact on the mass absorption coefficient. Three approximately 50:50 mixtures of Form I and Form II were prepared and analysed by XRPD. Expanded diffractograms of the pure Form I, Form II and a 50:50 mixture of forms are shown in Figure 2.

The areas of the Form I peaks and the Form II peak were measured using the Bruker X-ray diffraction evaluation software. From these areas and the known mass ratio of Form I and Form II, a response constant for Form I relative to Form II was calculated. This relative response constant was then used to confirm that a calibration curve using a range of Form I and II mixtures is linear and has a gradient of one and intercept of zero. The calibration curve of the calculated versus actual amount of Form I is shown in Figure 3 and agrees with the theory. The equation used to calculate the level of Form I in the mixture is:

$$\%\ \text{of Form I} = \frac{\text{Area I}}{\text{Area I} + (K \times \text{Area II})} \times 100$$

where Area I is the total area of Form I peaks at *c*. 7.5° 2θ, Area II is the area of the Form II peak at 8.0° 2θ and K is the response constant for Form I relative to Form II.

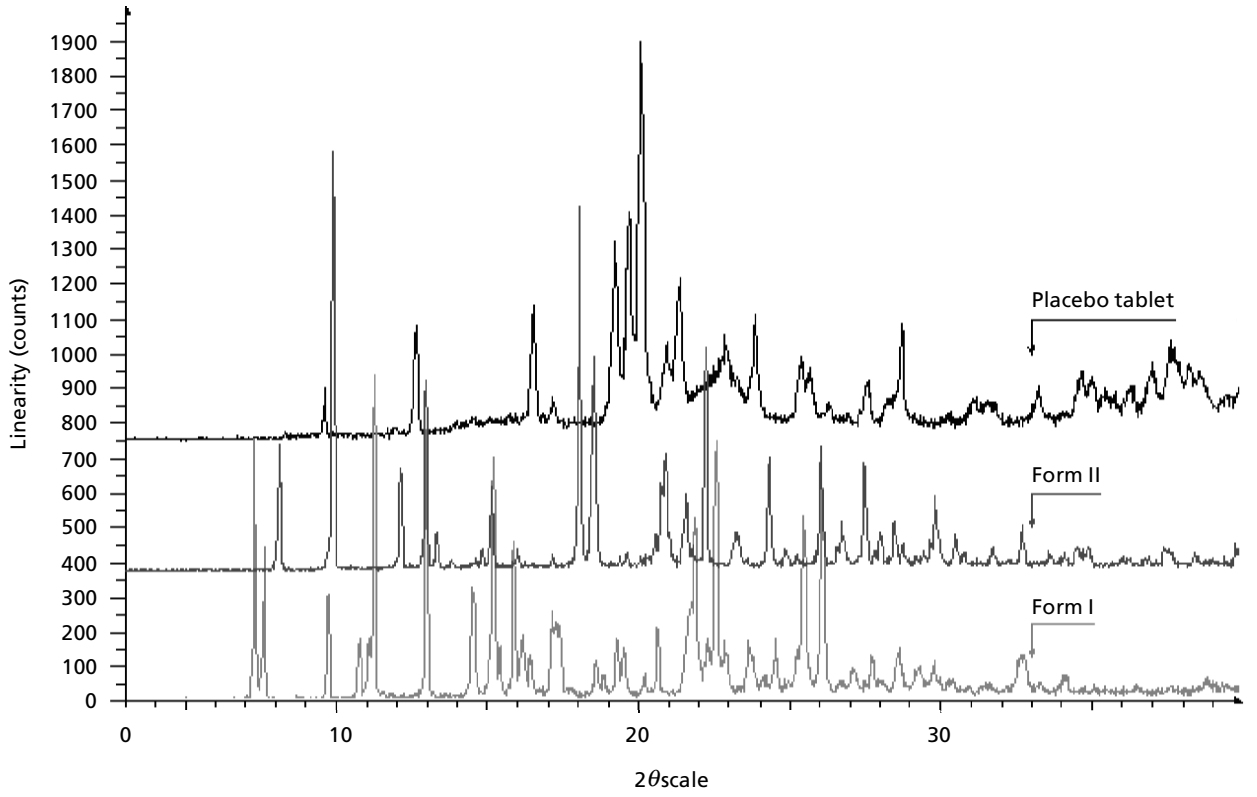

Figure 1 Comparison plot showing X-ray powder diffractograms of Form I, Form II and a sample of gently ground placebo tablet.

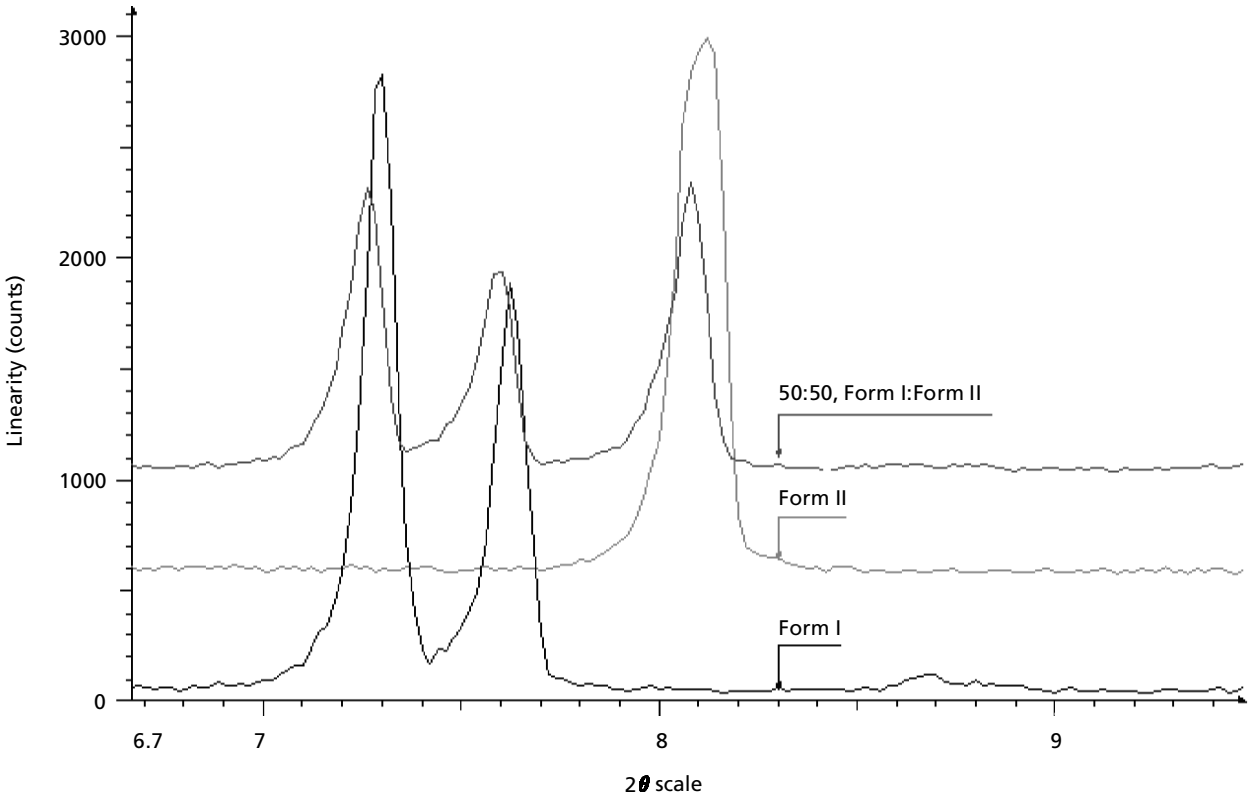

Figure 2 Comparison plot showing expanded X-ray powder diffractograms of Form I, Form II and a 50:50 mixture of the two forms.

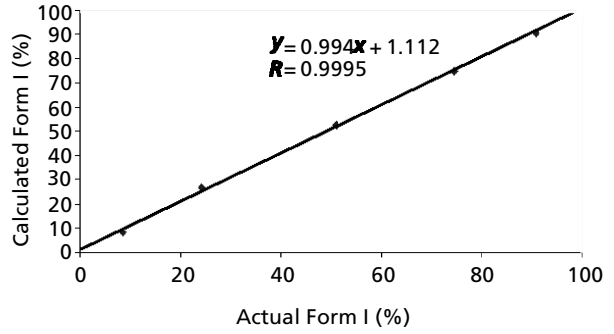

Figure 3 Linearity graph of the calculated versus actual amount of Form I demonstrating the linearity of the method in mixes of the API (n = 1). The residual standard deviation is 1.23.

Quantification in the formulated product

Once a method of polymorph quantification had been successfully developed and applied to the API, the method was transferred to the tablet formulation. The method provides a *relative* determination of Form I:Form II rather than an *absolute* determination. Hence, provided there is no excipient peak interference, the matrix in which the API is suspended should not affect the relative response constant previously determined. In order to confirm this, a series of API mixtures containing known ratios of the two forms was prepared. These were then blended with ground placebo tablets to give a mixture containing 25% w/w of the API. Again the calibration curve of calculated versus actual amount of Form I was linear with an intercept near zero and a gradient of one (Figure 4). As the two calibration curves both appeared to be acceptable within expected experimental errors, no further adjustment to the response constant K was performed.

It has been reported (Yamamura & Momose 2001) that APIs within tablet formulations can give rise to preferred orientation errors when analysed by XRPD, making quantitative measurements difficult. Although both Forms I and II were supplied with particle sizes of 95% < 25 μm, we would expect some errors to arise due to preferred orientation effects. Five repeat determinations of the 50:50 mix of Forms I and II in the simulated tablet showed some variation in the results: mean 50.7% Form I; relative standard deviation (RSD) 10.7%. This error could be increased to nearly 20% by preparing the sample on a flat silicon plate and smearing this with a glass slide. However, five repeat determinations of an actual tablet, which was found to contain approximately 40% Form I, gave excellent results: mean 39.5%; RSD 2.5%. Similar reproducibility was also obtained when the sample was prepared on the flat silicon plate. This would seem to indicate that preferred orientation and sample preparation are less of an issue in the final formulated product than in our modelled formulation. This indicates that for this product, the formulation process and subsequent sample preparation leads to essentially completely randomly orientated particles of API.

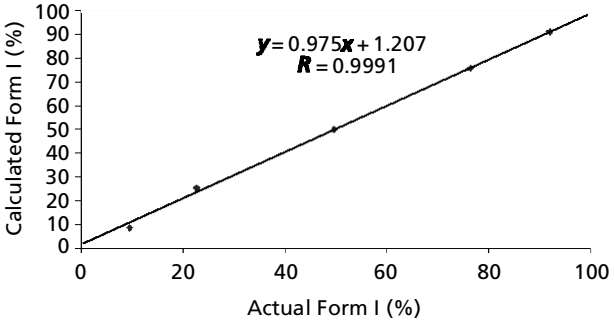

Figure 4 Linearity graph of the calculated versus actual amount of Form I demonstrating the linearity of the method in simulated tablet mixtures made from ground placebo tablets and API (25% w/w) (n = 1). The residual standard deviation is 1.67.

Crystalline form conversion in tablets

During the routine stability testing of the tablets stored at 40 °C/75% RH in open HDPE bottles the API was observed to convert from Form I to Form II. At all other stability conditions (5 °C/50% RH, 25 °C/60% RH, 30 °C/60% RH, 40 °C/20% RH in open HDPE bottles and 40 °C/75% RH in sealed HDPE bottles) no change in crystalline form was observed. These observations led to the conclusion that a combination of temperature and high humidity was required in order to effect the crystalline form change. Example diffractograms of tablets stored for 1 month at 40 °C/75% RH in open and sealed containers are shown in Figure 5.

Conversion of the unformulated API from Form I to Form II at 40 °C/75% RH in an open container does not occur. Form I does convert to Form II at 40 °C within a few hours if it is stirred in a 1% aqueous solution of sodium dodecyl sulfate (SDS), or over several days if stirred with water alone. These observations lead to the conclusion that water in contact with the API brings about form conversion at 40 °C. If this were true it would suggest that within the tablets stored at 40 °C/75% RH absorption of water by the formulation enables recrystallization of Form I to Form II. Some of the excipients within the formulation are hygroscopic and are likely to promote absorption of water at 40 °C/75% RH. It would be expected that these excipients are in close proximity to the API due to the granulation and compression processes used in the manufacture of the formulation. The excipients include a wetting agent, which would reduce the hydrophobicity of the API and hence increase its interaction with any absorbed water within the formulation, thereby aiding any potential recrystallization process. There is also the possibility that a small amount of crystal form conversion could occur during the tabletting process. This could occur during the wet granulation stage, during compression or during the film-coating. No evidence of form conversion was detected during any of these processes. However, if a small amount of crystals of API Form II were produced, these could seed a crystal form

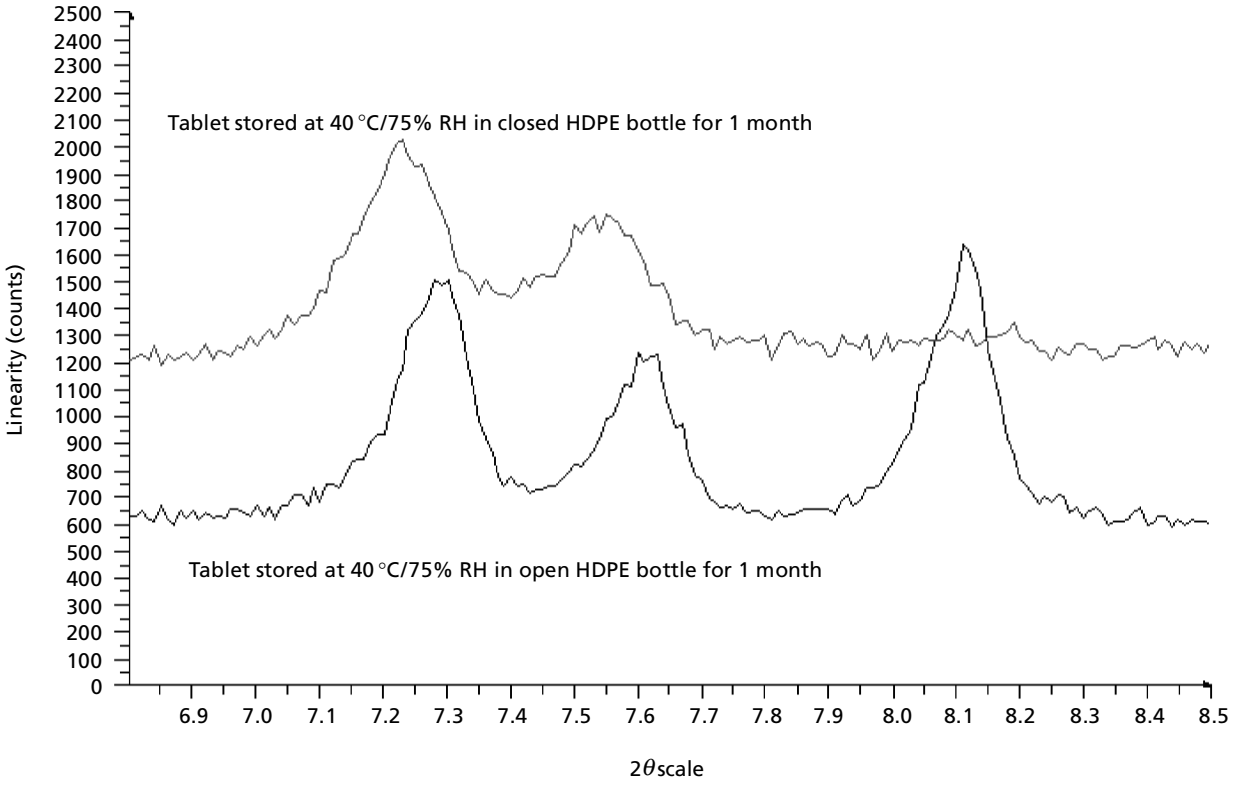

Figure 5 Comparison plot showing expanded X-ray powder diffractograms of gently ground tablets that were stored in open and closed HDPE bottles in a 40 °C/75% RH environmental cabinet.

conversion when the tablets are stored under high temperature and humidity conditions. This seeding process would be similar to that seen by Giron et al (1990), where the presence of 5% of a second polymorph brought about form conversion.

Form I, when in contact with water or under very high humidity conditions approaching 95% RH, has been shown to convert to a dihydrated form. This dihydrate formation is completely reversible if the water is removed or if the humidity drops back below a certain level (*c.* 70–80% RH depending on temperature). If the mechanism of conversion is via contact with water within the tablet, this conversion is likely to be via the intermediate dihydrate form.

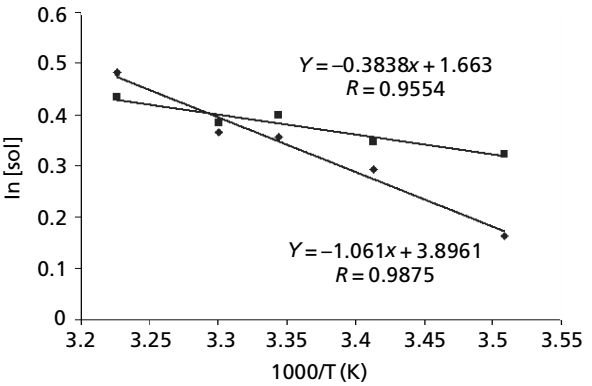

Figure 6 Van't Hoff plot showing solubility changes with temperature for Form I (◆; residual standard deviation 0.0215) and Form II (■; residual standard deviation 0.0131) in 1% aqueous SDS (n = 2).

Form I to Form II crystal form conversion

Form I was initially chosen as the form of API to be used in the formulated product because solubility measurements in organic solvent showed that at room temperature Form I had the lowest solubility and hence was the most thermodynamically stable crystalline form (Grant 1999). In order to investigate further the relative stability of the dihydrate formed from Form I and the tunnel hemihydrate Form II, the relative solubility of the two forms was measured in 1% aqueous SDS solution. A Van't Hoff plot was then drawn in a manner similar to that described by Brittain & Grant (1999) (Figure 6). It was shown that above the transition temperature T_t (*c.* 31 °C) Form II becomes the more thermodynamically stable crystal form, as it has the lower solubility. At 40 °C, as Form II is now more thermodynamically stable, a crystal form conversion can occur via recrystallization. This form conversion appears to be solubility driven and occurs over a few hours in 1% SDS, but only after several days in water where the solubility is very much less. If the temperature of the solution is lowered back to 25 °C the form conversion is reversed, with Form II converting back to Form I.

Observed form conversion in tablets

Tablets of Form I were manufactured and placed on stability at 40 °C/75% RH in open HDPE bottles. Tablets were removed periodically, gently ground and analysed by XRPD. The results of Form II content as a percentage compared with Form I were plotted. The tablets show detectable levels of Form II after only a few days. Form II content climbs slowly over the first few days before accelerating rapidly up to *c*. 43% after 13 days. After this point there is an inflection point and conversion slows and tends towards 100% Form II at T_{∞} (Figure 7). The initial slow conversion is presumably a lag time associated with the tablet reaching equilibrium and saturation with moisture in the high humidity condition. The slow rate of conversion in the latter stages may be due to mobility limitations of the water within the formulation. The relative rapidity of the conversion at the earlier stages also suggests that it is not a solid-state transformation, as such mechanisms would be expected to be very slow. The S-shaped curve observed in the conversion is very different from the conversion observed by Giron et al (1990), which showed an almost zero-order linear conversion with time.

Subsequently, if the tablets that had shown conversion are placed at a temperature below 31 °C, even under high humidity conditions, the Form II present in tablets does not convert back to Form I, even after months of storage. Reversal of conversion presumably does not occur because the exact conditions required in the tablet to reverse the transformation are not achieved. This would again seem to indicate that the mechanism of form conversion in the tablets is via recrystallization in regions in which the moisture content is sufficiently high to allow recrystallization to occur, facilitated by the hydrophobicity-lowering action of the excipients.

In order to provide further confirmation that form conversion is due to moisture ingress into the tablets, the outer surfaces of the tablets (*c*. 1 mm) were cut away and separated from the central core. The samples were then gently ground and analysed. The outer tablet surfaces were found to have much higher levels of Form II than the tablet cores, consistent with ingress of water from the surface of the tablet.

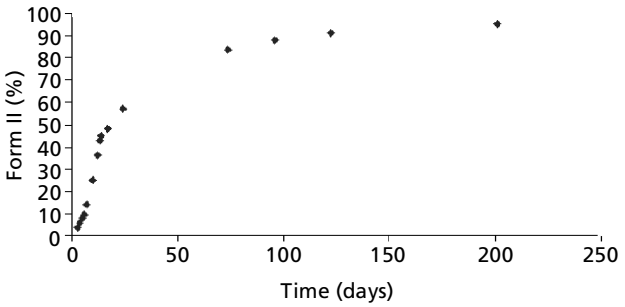

Figure 7 Graph showing percentage of Form II against time for tablets of Form I stored at 40 °C/75% RH in open HDPE bottles.

Conclusions

A crystalline form conversion has been observed in the API within tablets of a Merck development compound. This form conversion only occurs when tablets were stored under high humidity at 40 °C. The same conversion does not occur when the API alone is stored under this condition. When the API is stored at 40 °C in an aqueous environment the conversion is observed via recrystallization. This recrystallization process appears to proceed via an intermediate dihydrated form. The evidence would appear to indicate that inside the formulated tablet, excipients can interact with the API and moisture in such a way as to facilitate a crystalline form conversion. This observation emphasizes that stability of the API to form changes may not be predictive of stability of the API within the formulation. Once the API is incorporated into a formulated product its crystal form should be monitored closely under a variety of conditions. The micro-environment and associated interactions within a tablet may be very different from those of the surrounding atmospheric environment.

A quick and easy method for quantifying the ratios of the two forms in the tablets was developed using XRPD. Form conversion was monitored in tablets stored at 40 °C/75% RH in open HDPE bottles. 50% conversion from Form I to Form II occurs after only three weeks, but complete conversion to 100% Form II would require a much longer time period. Although the possibility of crystalline form conversion of API within a formulation is often mentioned in publications, there are few published occurrences. By monitoring the crystal form of the API in the tablets under a variety of stability conditions, it quickly became apparent that protection from moisture or reformulation would be required to ensure that form conversion did not occur in the final product under acceptable storage conditions.

References

Agatonovic-Kustrin, S., Tucker, I. G., Schmierer, D. (1999) Solid state assay of ranitidine HCl as a bulk drug and as active ingredient in tablets using DRIFT spectroscopy with artificial neural networks. *Pharm. Res.* **16/9**: 1477–1482

Brittain, H. G., Grant, D. J. W. (1999) Effects of polymorphism and solid-state solvation on solubility and dissolution rate. In: Brittain, H. G. (ed.) *Polymorphism in pharmaceutical solids*. 1st edn, Marcel Dekker, New York, pp 284–289

Chao, R., Vail, K. C. (1987) Polymorphism of 1,2-dihydro-6-neopentyl-2-oxonitic acid: characterization, interconversion and quantitation. *Pharm. Res.* **4/5**: 429–432

Findlay, W. P., Bugay, D. E. (1998) Utilization of Fourier transform-Raman spectroscopy for the study of pharmaceutical crystal forms. *J. Pharm. Biomed. Anal.* **16**: 921–930

Gao, P. (1996) Determination of the composition of delavirdine mesylate polymorph and pseudopolymorph mixtures using 13C CP/MAS NMR. *Pharm. Res.* **13/7**: 1095–1104

Gimet, R., Luong, A. T. (1987) Quantitative determination of polymorphic forms in a formulation matrix using the near infrared reflectance analysis technique. *J. Pharm. Biomed. Anal.* **5**: 205–211

Giron, D., Goldbronn, C. (1997) Use of DSC and TG for the identification and quantification of the dosage form. *J. Therm. Anal*. **48**: 473–483

Giron, D., Edel, B., Piechon, P. (1990) X-ray quantitative determination of polymorphism in pharmaceuticals. *Mol. Cryst. Liq. Cryst*. **187**: 297–307

Grant, D.J.W. (1999) Theory and origin of polymorphism. In: Brittain, H. G. (ed.) *Polymorphism in pharmaceutical solids*. 1st edn, Marcel Dekker, New York, pp 10–18

Haleblian, J. (1975) Characterisation of habits and crystalline modification of solids and their pharmaceutical applications. *J. Pharm. Sci*. **64**: 1269–1288

Haleblian, J., McCrone, W. (1969) Pharmaceutical applications of polymorphism. *J. Pharm. Sci*. **66**: 669–673

Patel, A. D., Luner, P. E., Kemper, M. S. (2000) Quantitative analysis of polymorphs in binary and multi-component powder mixtures by near-infrared reflectance spectroscopy. *Int. J. Pharm*. **206**: 63–74

Saindon, P. J., Cauchon, N. S., Sutton, P. A., Chang, C., Peck, G. E., Byrn, S. R. (1993) Solid-state nuclear magnetic resonance (NMR) spectra of pharmaceutical dosage forms. *Pharm. Res*. **10/2**: 197–203

Suryanarayanan, R., Herman, C. S. (1991) Quantitative analysis of the active ingredient in a multi-component tablet formulation by powder X-ray diffractometry. *Int. J. Pharm*. **77**: 287–295

Yamamura, S., Momose, Y. (2001) Quantitative analysis of crystalline pharmaceuticals in powders and tablets by a pattern-fitting procedure using X-ray powder diffraction data. *Int. J. Pharm*. **212**: 203–212