

**WETENSKAPLIKE BYDRAES**  
**REEKS H: INTREEREDE NR. 189**

**CRYSTAL POLYMORPHISM AND ITS OCCURANCE AMONG ACTIVE  
PHARMACEUTICAL INGREDIENTS IN SOUTH AFRICA**

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**Intreerede gehou op 6 Mei 2005**

Die Universiteit is nie vir menings in die publikasie aanspreeklik nie.

Navrae in verband met *Wetenskaplike Bydraes* moet gerig word aan:

Die Registrateur  
Noordwes-Universiteit  
2520 POTCHEFSTROOM

Kopiereg © 2005 NWU

**ISBN** 1-86822-508-9

## **CRYSTAL POLYMORPHISM AND ITS OCCURANCE AMONG ACTIVE PHARMACEUTICAL INGREDIENTS IN SOUTH AFRICA**

### **Introduction and background**

In South Africa, the pressure to provide more affordable medicines has dramatically increased the manufacturing and marketing of generic equivalents. Given the large selection of suppliers it is sometimes difficult to choose materials with the correct profiles regarding purity and physico-chemical properties. However, cheaper, or more affordable drugs, does not mean that their quality, effectiveness and safety should be sacrificed (Videau, 2001).

The replacement of an active pharmaceutical ingredient (API) with its generic requires the approval thereof, based on the outcomes of a range of comparative tests that are being performed (Videau, 2001).

Many pharmaceutical solids exhibit polymorphism. Polymorphism is frequently defined as the ability of a substance to exist as two or more crystalline phases that have different arrangements and / or conformations of the molecules in the crystal lattice. As a result, polymorphic solids have different unit cells and display different physical properties, such as density, hardness, tableting ability, melting point, solubility and dissolution rate (Vippagunta *et al.*, 2001).

Current research emphasises the importance of controlling the crystal form of the active pharmaceutical ingredient during the different phases of manufacture. Any phase transition of the crystal form during manufacture may affect the bioavailability of the active pharmaceutical ingredient.

Some of the most recent studies on active pharmaceutical ingredients (APIs) available on the South African market, where polymorphism was identified, are discussed.

### **Polymorphism and pseudopolymorphism in active pharmaceutical ingredients**

Solids may exist as crystalline solids and amorphous materials (figure 1). A crystalline solid has a well defined structure and melting point, whilst an amorphous material has no well defined molecular structure, and because of its distinctive properties it is

sometimes regarded as a polymorph (Grant, 1999). The most common forms for crystalline materials are polymorphs and pseudopolymorphs (Vippagunta *et al.*, 2001).

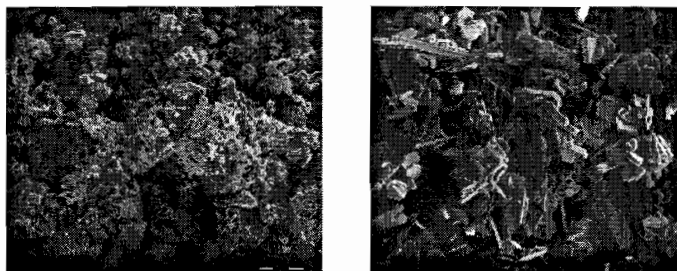


Figure 1 Amorphous (left) and crystalline (right) forms of clarithromycin (De Jager, 2005).

The term polymorphism comes from the Greek words *poly* = many, *morph* = form (Bernstein, 2002). Crystalline polymorphs have the same chemical composition, but different internal structures and therefore different physico-chemical properties. The different crystal structures arise when the API crystallises in different crystal packing arrangements and/or different conformations (Vippagunta *et al.*, 2001). Solvates, also called pseudopolymorphs, are crystalline solid adducts containing solvent molecules within its crystal structure. If the incorporated solvent is water, it is called a hydrate (Vippagunta *et al.*, 2001). Polymorphs and pseudopolymorphs differ in crystal packings, causing differences in their physical properties, such as densities, hardness, tableting ability, refractive index, melting point, solubility and dissolution rates (Grant, 1999).

As was mentioned, current research emphasises the importance of controlling the crystal form of the API during the different phases of manufacture. Phase transitions, such as polymorph interconversion, desolvation of a solvate, hydrate formation and conversion of a crystalline material into an amorphous form may occur during various pharmaceutical processes (Vippagunta *et al.*, 2001). In this regard, Brittain & Fiese (1999), for example, discussed the unintentional conversion of polymorphs and the desolvation of hydrates upon exposure to the energetics of pharmaceutical processing, since, according to them, conditions as harsh as 80°C and 100% RH for up to 6 hours

are not unusual during the routine manufacture of dosage forms. They (Brittain & Fiese, 1999) pointed out that a variety of phase conversions were possible upon exposure to the energetic steps of bulk material storage, drying, milling, wet granulation, oven drying and compaction.

Of importance is that any phase transition of the crystal form during manufacture may affect the bioavailability of the API. It is thus preferable to select the most stable form before starting with manufacture and to control the crystal form during the whole developmental process (Vippagunta *et al.*, 2001). The presence of a metastable form during formulation, manufacture, or in the final dosage form may often lead to instability with drug release, due to phase transitions (Borka, 1991; Rodriguez-Hornedo *et al.*, 1992). In suspensions, the use of the wrong polymorph may cause a phase transition from metastable to stable with subsequent crystal growth (Borka, 1991). The higher solubility of the metastable form of digoxin resulted in overdosing, until its solid transfer behaviour was established (Brittain & Grant, 1999).

Solutions are normally independent of polymorphic problems, but if there should happen to exist a less soluble form should occur, it would become known upon stability testing. Brittain and Fiese (1999) warned that temperature cycling poses the most severe challenge to solubility and if one should generate seed crystals of a less soluble form of a compound during cooling, then equilibrium is rapidly established, which could result in precipitation or crystal growth. An example of this scenario is in the industry where the soluble anhydrous material converts into an insoluble hydrate upon stability testing (Brittain & Fiese, 1999).

### **Preparation of polymorphic forms**

It still remains one of the largest challenges to predict the number of polymorphic forms that a drug may have (Vippagunta *et al.*, 2001). The use of computer technology allows for the prediction of possible polymorphic forms based on molecular structure, however computational methods for theoretically predicting polymorphic forms have many limitations (Vippagunta *et al.*, 2001).

Slow solvent evaporation is a valuable method for producing crystals. The solvents selected for recrystallisation should include those, which the API would come into

contact with during synthesis, purification and processing, as well as solvents having a range of boiling points and polarities (Guillory, 1999). If polymorphs exist, it is necessary to examine the physical properties of the different polymorphs, i.e. solubility, stability, crystal morphology and thermal properties (Byrn *et al.*, 1995).

### **Characterisation of pharmaceutical solids**

The field of solid-state chemistry of APIs and drug excipients includes many scientific disciplines. Fortunately, the variety of available characterisation methods makes it possible to detect virtually any problem that could be encountered during the course of drug development (Vippagunta *et al.*, 2001). Techniques used to investigate the APIs included X-ray powder diffraction (XRPD), single crystal X-ray structure determination, differential scanning calorimetry (DSC), thermogravimetric analysis (TA), infrared spectroscopy (IR), particle size analysis, electron microscopy, dissolution, solubility determinations and hot stage microscopy.

Hot-stage microscopy provides a rapid and effective method for screening APIs for the existence of polymorphism. Solvates and hydrates may be readily detected, since desolvation and dehydration can be observed by covering the API with silicone oil which trap the released solvent (Bernstein, 2002).

X-ray powder diffraction is a powerful tool for the investigation of crystalline solids. This method is experimentally simple and does not require large single crystals, but instead can rapidly be applied to any powdered sample. Modern diffractometers can be fitted with an environmental chamber that allows control of the temperature. This method, variable temperature X-ray powder diffractometry (VTXRPD) has been quite useful in the study of transformations and interconversions of crystal forms, desolvations and other processes (Byrn *et al.*, 1999).

Single X-ray crystallography is an excellent tool for the investigation of crystalline solids. In most cases it can lead to the complete determination of the structure of the solid, as well as the determination of the crystal packing relationship among individual molecules in the solid (Byrn *et al.*, 1999).

The most important thermal methods for the study of solid-state chemistry are thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC). TGA

measures the change in the mass of the sample as temperature is changed. DSC involves measuring and comparing the melting points of the sample with a reference compound (Byrn *et al.*, 1999).

The dissolution rate and the solubility of solids in water or other solvents are important aspects of the solid-state chemistry of drugs, as they can differ for different polymorphs, solvates, hydrates, anhydrous and amorphous forms of the same API (Byrn *et al.*, 1999).

Interest in particle size analysis can be attributed to an increasing awareness of its applicability to a number of practical problems. Bulk properties, such as bulk density, flowability, mixing ability and segregation of mixed materials are related to particle size (Byrn *et al.*, 1999).

Infrared spectroscopy (IR) is very useful for the analysis of solids (Byrn *et al.*, 1999). In the technique of diffuse reflectance infrared spectroscopy (DRIFTS) the sample is usually dispersed in powdered potassium bromide, a procedure which is ideal for studying polymorphic forms in APIs. This technique is less likely to lead to polymorphic transformations or loss of solvent than the more aggressive grinding required in making a pellet (Roston, 1993). The infrared spectrum is extremely sensitive to the structure, conformation and environment of an organic compound and thus is a powerful method for the characterisation and identification of different solid forms of drugs (Byrn *et al.*, 1999).

### **Differences found among physico-chemical properties of generic APIs in South Africa**

Important parameters in the quality of raw materials include physical characteristics that affect the bioavailability of the finished product. It has clearly been demonstrated that the polymorphic state of the active drug substance can affect the bioavailability of the finished product. Toxic effects may also be linked to polymorphism (e.g. mebendazole). The size and morphology of particles may give different rates of dissolution (aspirin, paracetamol, etc.) (Andriolli *et al.*, 1998).

Some of the most recent studies on active pharmaceutical ingredients that are available on the South African market, where polymorphism was identified, are

discussed. The aim of this discussion is to demonstrate how different polymorphic forms of a given API resulted in different physico-chemical results. In the case of mebendazole and rifampicin the dissolution rates of the different polymorphic forms differed substantially.

### **Mebendazole**

Mebendazole, a broad spectrum anthelmintic drug. It is practically insoluble in water and studies of its polymorphism has led to the identification and characterization of three polymorphic forms, A, B, and C, displaying solubility and therapeutic differences that show that polymorph C is pharmaceutically favoured. The objective of this study was to adjust the USP dissolution test for mebendazole so that it was able to distinguish between the dissolution properties of three mebendazole polymorphs. This would provide generic manufacturers with one more tests to ensure that the therapeutically active polymorph C is used. The results obtained in this study showed that the USP dissolution test conditions were unable to distinguish between the dissolution properties of completely dispersed mebendazole polymorphs having comparable particle sizes. When sodium lauryl sulphate was removed from the dissolution medium, the percentage dissolved *versus* time profiles, changed so that polymorph C dissolved faster (70% within 120 minutes) compared to polymorph B (37% within 120 minutes) and polymorph A (20% within 120 minutes). The polymorphs differed with respect to their X-ray powder diffractograms (figure 2), IR spectra (Table 1) and the differences in morphology could be observed by means of SEM photos (figure 3) (Liebenberg *et al.*, 1998; Swanepoel *et al.*, 2003a; Swanepoel *et al.*, 2003b).



### XRPD Mebendazole Polymorphs

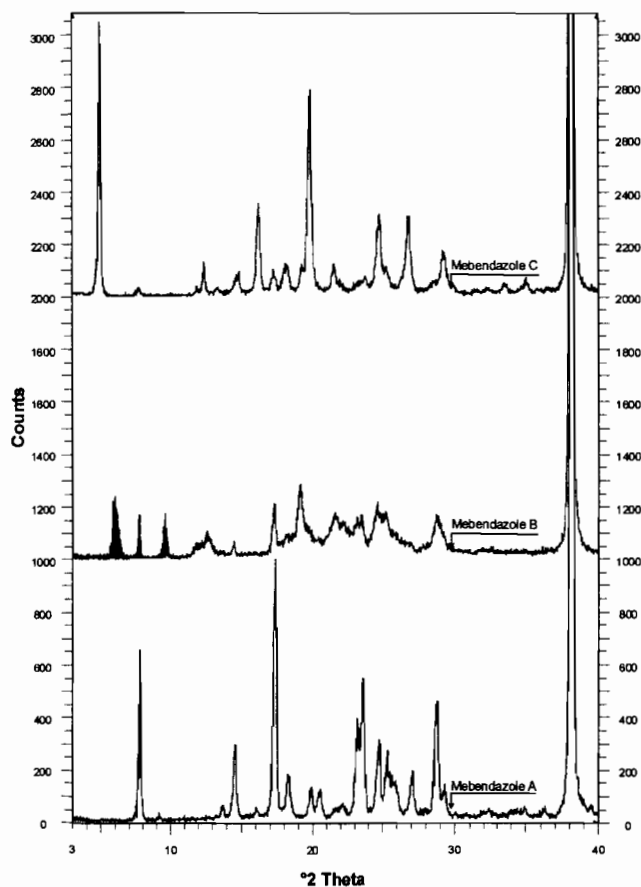
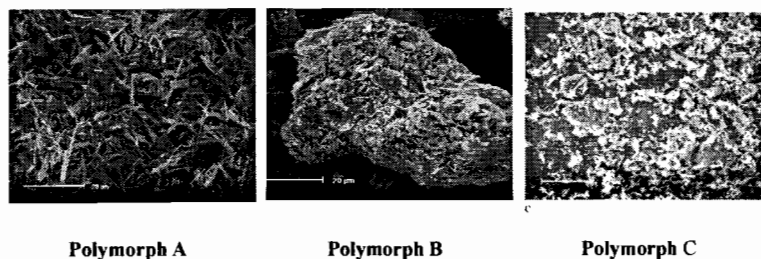


Figure 2 XRPD patterns of mebendazole polymorphic forms A, B, C (Swanepoel *et al.*, 2003a).

**Table 1 Main absorbencies in the Fourier transform IR spectra of the mebendazole polymorphs**

Crystal form	-NH (cm <sup>-1</sup> )	-C=O (cm <sup>-1</sup> )
Form A	3370	1730
Form B	3340	1700
Form C	3410	1720



**Figure 3 SEM photos of mebendazole polymorphic forms A, B and C (Swanepoel *et al.*, 2003a).**

According to the USP dissolution test for mebendazole, not less than 75% (Q) of the labeled amount of the drug must dissolve in 120 minutes from 6 individual tablets, in 900 ml of a 0.1 M hydrochloric acid solution containing 1% sodium lauryl sulfate, a surface active agent. The results obtained in this study showed that these test conditions were unable to distinguish between the differences in the dissolution properties of completely dispersed mebendazole polymorphs with comparable particle sizes. Solubility studies in 0.1 M HCl have shown the solubility of mebendazole to be very low and in the order A<C<B (Costa *et al.*, 1991). Since more than 75% of the polymorphs dissolved in 120 minutes, all within the USP tolerance, the dissolution properties of the powders were equal in the USP medium. Under these conditions, increased solubility, due to the presence of sodium lauryl sulfate, dominates the dissolution rate, and differences in the dissolution rate were eliminated because

sodium lauryl sulfate enhanced the solubility of this poorly water-soluble drug due to wetting, micellar solubilisation, and/or deflocculation. However, for mebendazole the sodium lauryl sulfate present in the dissolution medium reduced the ability of the test to distinguish between the three polymorphic forms of mebendazole (Swanepoel *et al.*, 2003a).

When sodium lauryl sulfate was removed from the dissolution medium, the percentage dissolved *versus* time profiles, changed dramatically. Now it was clear that polymorph C went into solution faster (70% in 120 minutes), compared to polymorph B (37% in 120 minutes) and A (20% in 120 minutes). This order in the dissolution rate ( $A < B < C$ ) did not correlate with the reported differences in solubility but correlated with the reported *in vivo* effectiveness of the polymorphs (Rodriguez-Caabeiro *et al.*, 1987; Costa *et al.*, 1991; Charoenlarp *et al.*, 1993). This suggested that the dissolution rate of the polymorphs depended on more than just the inherent solubility of each polymorph and the degree of dispersion of the drug in the medium in which it was dissolving.

The dissolution rates of the three forms, as dispersed powders with particle sizes below 10  $\mu\text{m}$ , were measured according to the method of the USP. The dissolution medium was 0.1 M HCl containing 1% sodium lauryl sulphate and the dissolution profiles obtained therein are shown in figures 4-6. These figures also show the dissolution in 0.1 M HCl without surfactant and the effect of the surfactant concentration on dissolution when the concentration of sodium lauryl sulphate was varied from 0.1 – 1% (Swanepoel *et al.*, 2003a).

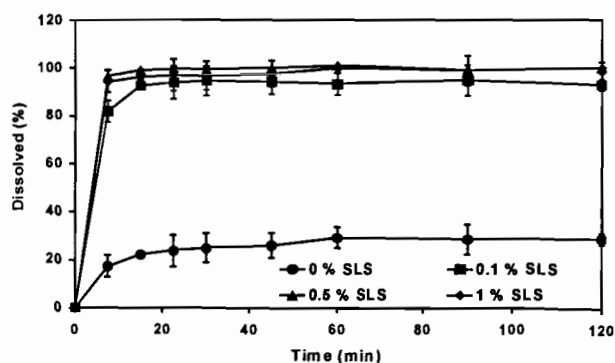


Figure 4 Powder dissolution profiles of form A (Swanepoel *et al.*, 2003a).

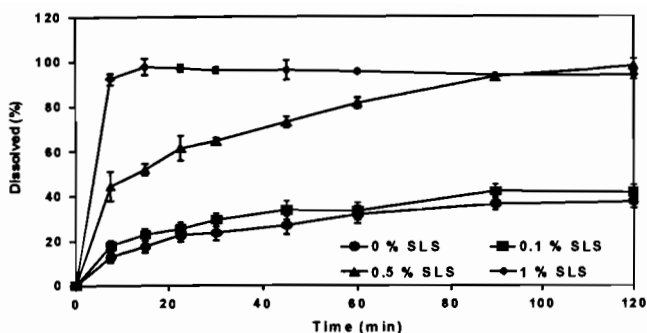


Figure 5 Powder dissolution profiles of form B (Swanepoel *et al.*, 2003a).

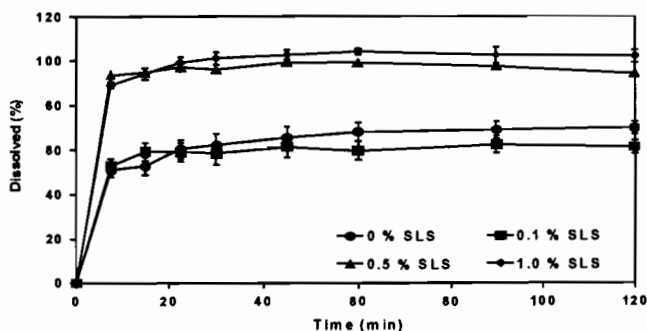


Figure 6 Powder dissolution profiles of form C (Swanepoel *et al.*, 2003a).

According to the USP not less than 75% (Q) of the drug must be dissolved within 120 min (figure 7). In the USP medium all three polymorphs dissolved more than 75% within 120 min., Form C = 102% > Form A = 95% > Form B = 94%. In 0.1 M HCl the dissolution rates were significantly lower, but this medium distinguished between the differences in the solubility of the three forms, Form C = 72% > Form B = 45% > Form A = 20%. By increasing the concentration of sodium lauryl sulphate in the dissolution medium the discriminating power of the medium was diminished (Swanepoel *et al.*, 2003a).

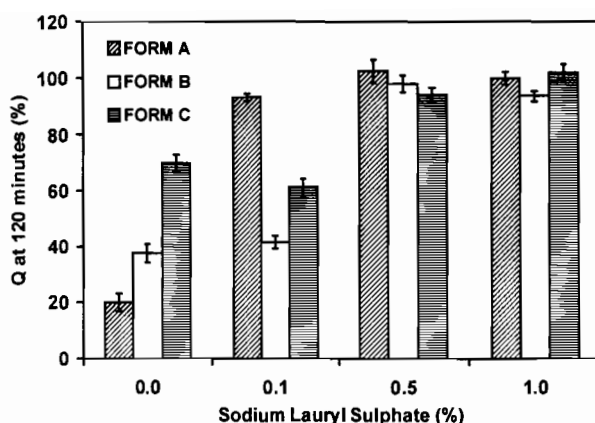


Figure 7 Effect of sodium lauryl sulfate concentration on Q at 120 min (Swanepoel *et al.*, 2003a).

Manufacturers and regulatory agencies should be aware when buying or sourcing mebendazole raw material, tablets or suspensions, since dissolution results obtained using the USP conditions would not ensure that the products contain the preferred polymorph C. This is important, since all three polymorphic forms of mebendazole are found on the market (Liebenberg *et al.*, 1998). In developing countries such as South Africa, there are numerous generic mebendazole products available and these products are widely used, since the drug forms an integral part of the essential drug list in this country. Consideration should therefore be given to eliminating sodium lauryl sulfate from the dissolution medium for mebendazole, because it will increase the ability of the dissolution test to discriminate between mebendazole polymorphs. Furthermore, other tests, including IR analysis and X-ray powder diffractometry should also be used to ensure that the therapeutically preferred mebendazole polymorph C is present in drug products.

## Rifampicin

Rifampicin is a major drug of choice in the treatment of tuberculosis and leprosy. Rifampicin shows polymorphism, which makes it necessary to select a suitable crystal form at an early stage of development to ensure optimum solubility and dissolution rates. Three solid forms were identified, i.e. Forms I and II, and an amorphous form. Commercially available materials mainly consist of Form II and a mixture of Form II and the amorphous form (Henwood *et al.*, 2000).

This study was prompted by several failures in dissolution equivalency of rifampicin products manufactured in South Africa. On visual inspection of the raw material samples, it was evident that the powders had definite differences in their particle sizes and shapes. This led to the investigation into the crystal properties of several rifampicin raw materials available to manufacturers in South Africa (Henwood *et al.*, 2000).

Visual inspection of the raw materials with SEM (figure 8) showed definite differences in their particle sizes and shapes. Crystals of powder A, with a mean particle size of 164  $\mu\text{m}$  were “bricklike” to elongated with even sides. The particles of powder B were not well defined, but had a smooth surface, with a mean particle size of 107  $\mu\text{m}$ . Sample C was a mixture of rod-like to shapeless particles with a mean diameter of 95  $\mu\text{m}$ . Particles of sample D were characterised by uneven surfaces and a mean particle size of 147  $\mu\text{m}$ . The crystals of sample E were rod-like with a mean particle size of 170  $\mu\text{m}$  (Henwood *et al.*, 2000).

The best indication of polymorphism was differences in the XRPD patterns (figure 9) of the powders. Comparison with XRPD patterns reported by Pelizza *et al.* (1977), indicated that powders A, B and E were the same as Form II. The main characteristics of Form II were present in powders C and D. However, a sharp drop in intensity counts from about 6400 to 1225 indicated that a large percentage of a less crystalline form was present in these powders. Most probably, powders C and D were mixtures of an amorphous form and Form II (Henwood *et al.*, 2000).

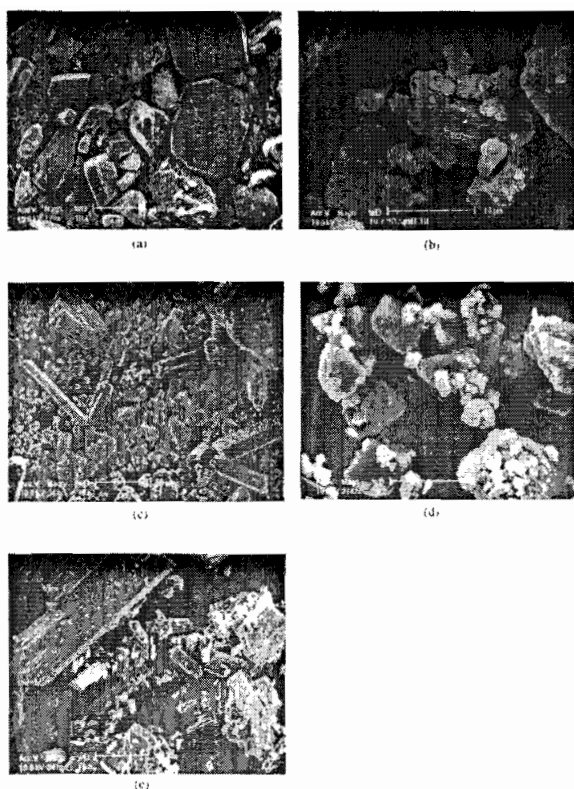


Figure 8 Photomicrographs of rifampicin powders (Henwood *et al.*, 2000).

The dissolution profiles of the powders in 0.1 M HCl, buffer pH 7.4 and water are shown in figures 10-12. Similarity factors  $f_2$  being calculated showed that the dissolution profiles of rifampicin powders in 0.1 M HCl were within 10% of each other and therefore similar. In buffer pH 7.4, powders A, B and E, and powders C and D, had similar dissolution profiles, respectively, but the profiles of powders C and D were not similar to those of A, B and E. The slower dissolutions of powders C and D were even more pronounced in water. This result was unexpected since it is generally thought that amorphous materials are more soluble than crystalline materials (Henwood *et al.*, 2000).

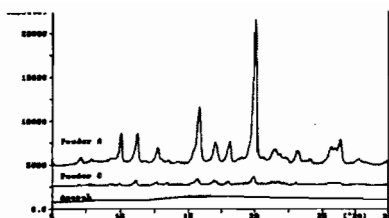


Figure 9 XRPD diffractograms of powders A and C (Henwood *et al.*, 2000).

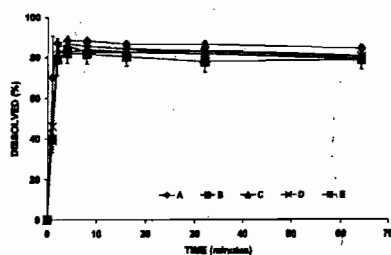


Figure 10 Dissolution profiles of the different rifampicin powders in 0.1 M HCl (Henwood *et al.*, 2000).

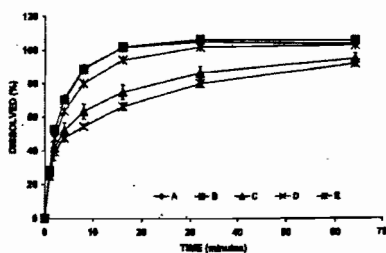


Figure 11 Dissolution profiles of the different rifampicin powders in phosphate buffer pH 7.4 (Henwood *et al.*, 2000).



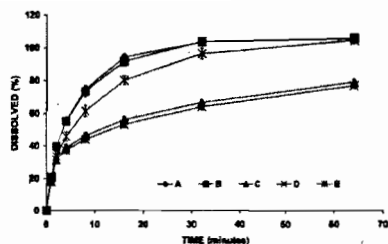


Figure 12 Dissolution profiles of the different rifampicin powders in water (Henwood *et al.*, 2000).

The results of this study showed that the main difference among the powders was the amorphous content. The presence of amorphous rifampicin could be detected by XRPD, IR and DSC methods. The dissolution rates of the different rifampicin powders did not differ in 0.1 M HCl. The presence of amorphous materials slowed the dissolution rate in water and buffer pH 7.4. This behaviour was attributed to the electrostatic properties of the very fine particles in the amorphous powders. Electrostatic forces resulted in lump formation, which was observed during dissolution testing (Henwood *et al.*, 2000).

The following examples of different polymorphic forms identified amongst APIs will be discussed, i.e. venlafaxine HCl, spironolactone, zopiclone, roxithromycin and clarithromycin. These examples illustrate the different characterisation methods to identify and characterise different polymorphic forms.

### Venlafaxine HCl

Venlafaxine hydrochloride structurally is a novel phenethylamine antidepressant, which inhibits monoamine re-uptake, with the greatest effect on serotonin, a substantial effect on norepinephrine, and relatively minor effects on dopamine (Potter *et al.*, 1998). Raw materials of venlafaxine hydrochloride, available on the market, were investigated. Two forms were identified, as well as a mixture of the two forms.

The different polymorphic forms were identified by means of a XRPD study (Brits, 2003).

The X-ray powder diffractograms of two of the four samples (samples 1 and 4) were identical. The X-ray powder diffractogram of sample 2 differed from those of samples 1 and 4. This was reflected by the presence of additional peaks at 8.4, 12.7 and 21.2°2 $\theta$ , and the absence of peaks at 22.6, 29.3 and 32.2°2 $\theta$ . All the peaks of sample 3 corresponded with those of samples 1 and 4. However, an additional peak at 12.7°2 $\theta$  was present in sample 3. Figure 13 is an overlay of the X-ray powder diffractograms of the four samples, which illustrates the differences obtained (Brits, 2003).

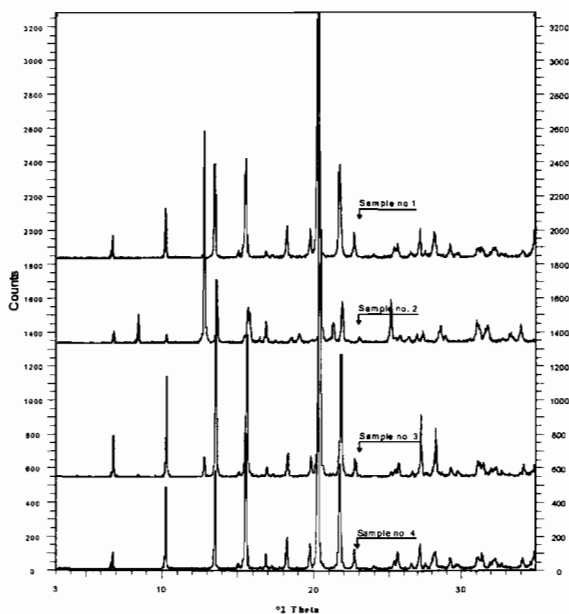


Figure 13 X-ray powder diffractogram overlay of the venlafaxine HCl samples (Brits, 2003).

This study confirmed that two of the four venlafaxine HCl samples (samples 1 and 4) were identical with respect to chemical structure and polymorphic modification. Although the IR spectra showed that samples 1, 3 and 4 were identical, XRPD data showed that samples 2 and 3 were probably mixtures of polymorphic forms (Brits, 2003).

### Spironolactone

Spironolactone is a diuretic steroidal aldosterone agonist known to show variable and incomplete oral behaviour because of poor water solubility and dissolution rate (Aganof *et al.*, 1991). This might be due to variations in the crystal form, since the crystal properties of spironolactone are complex; as it can adopt polymorphic, non-stoichiometrically solvated, or amorphous glass forms from the same solvents, and can undergo solvent mediated and other solid-state transformations (Aganof *et al.*, 1991; Salole *et al.*, 1985; El-Dash *et al.*, 1983). This study reported the usefulness of variable temperature X-ray powder diffractometry (VTXRPD) as a fast method to characterise and measure the transformation between two spironolactone polymorphs, and mixtures thereof, found among raw material samples randomly obtained from pharmaceutical bulk suppliers (Liebenberg *et al.*, 2003).

In this study, the physicochemical properties of five randomly obtained samples of spironolactone were determined. The median particle sizes by volume of all the samples were identical and small,  $\leq 6 \mu\text{m}$ . There were no significant differences in the dissolution of the powders in three dissolution media (0.1 M HCl + 0.1% SLS; 0.1 M HCl; and water). However, the dissolution rates in the three media decreased in the order 0.1 M HCl + 0.1% SLS > 0.1 M HCl > water. In water and 0.1 M HCl, only 17% and 16% dissolved after 60 minutes for both the stable and the metastable forms, respectively. DSC analysis showed that samples 1 and 2 exhibited a single melting endotherm at 204°C and samples 3, 4 and 5 at 205-206°C. No additional crystal transformations, other than the melting process, were observed. These results were not in line with the reported melting points of the crystal forms and at first glance suggested that the samples contained the same crystal form. However, small differences in the DRIFTS spectra of the two groups of powders suggested the

presence of some impurities (residual solvents), or polymorphic mixtures (Liebenberg *et al.*, 2003).

According to XRPD data, figures 14 and 15, the five samples represented two distinctive groups of spironolactone powders. Based on the X-ray diffraction data for the different crystal forms of spironolactone, as reported by Aganofov *et al.* (1991), samples 3, 4 and 5 were the same as the thermodynamically stable form obtained from acetone, i.e. Form II. Figure 14 represents the XRPD patterns of sample 3 when exposed to an increase in temperature. These samples did not show any change in crystal form upon heating up to 195°C and represented pure samples of Form II, characterised by a singlet at  $9.2^{\circ}2\theta$ , a doublet at  $11.6$  and  $12.2^{\circ}2\theta$ , and a triplet at  $16.1$ ,  $16.8$  and  $17.3^{\circ}2\theta$  in the XRPD pattern. The XRPD patterns of samples 1 and 2 were different from that of the thermodynamically stable Form II. Careful analysis of the XRPD patterns (figure 15) of these powders showed that the samples were mixtures of Forms I and II. Both the main peaks mentioned above for Form II and those characteristic for Form I ( $13.2$ ,  $14.6$ ,  $15.2$ , and  $17.6^{\circ}2\theta$ ) were present in the XRPD patterns of these samples. Further analysis of the XRPD patterns showed that these powders contained between 20-50% of Form I. Previously another sample from the same supplier of sample 2 spontaneously transformed into Form II when stored at room temperature. Upon heating, figure 15, the mixture also completely transformed into Form II. The change was gradual in the temperature range from 25-75°C. As the temperature increased above 100°C, samples 1 and 2 were quickly transformed into Form II. This polymorphic change was evident from the disappearance of the peaks at  $13.2 - 15.2^{\circ}2\theta$ . The XRPD pattern at 175°C also matched that of Form II shown in figure 14. This result was contradictory to previous reports that Form I and II are monotropic crystal forms that don't change into each other upon heating (Liebenberg *et al.*, 2003).

This comparative raw material characterisation study confirmed that spironolactone exists as different crystal forms, predominantly the thermodynamically stable Form II, as well as mixtures of this form and a metastable Form I. Out of five samples tested, three were Form II and two a mixture of Form I and II. Mixtures of the intermediate metastable form and the stable form of spironolactone had comparable melting points and DSC analysis could therefore not be used to determine the polymorphic purity of

the samples. IR analysis and dissolution testing were also unable to distinguish between the crystal forms. VTXRPD proved to be very useful in establishing the polymorphic purity of the samples (10). It also conclusively showed that Form I, the metastable form, transformed into Form II the thermodynamically stable form. This change was more rapid at higher temperatures (Liebenberg *et al.*, 2003).

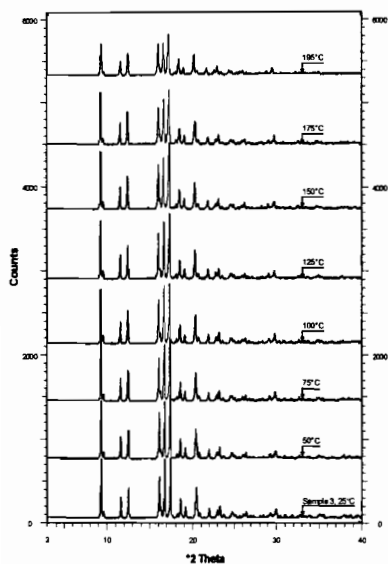
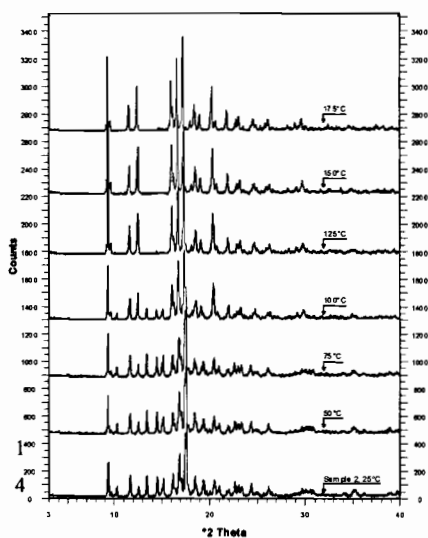


Figure 14

Figure 15

Figure



Variable temperature XRPD patterns of spironolactone sample 3, representing the thermodynamically stable crystal Form II (Liebenberg *et al.*, 2003).

Figure 15 Variable temperature XRPD patterns of spironolactone sample 2, characterising the phase changes upon heating of a powder containing a mixture of Forms I and II (Liebenberg *et al.*, 2003).

## Zopiclone

Zopiclone is a cyclopyrrolone drug with sedative and hypnotic properties. It is chemically unrelated to the benzodiazepines, but has a similar spectrum of activities; it binds to sites on or closely linked to the benzodiazepine receptor complex (Goa & Heel, 1986). A range of characterisation methods was used to characterise the crystal properties of zopiclone powders obtained from different suppliers. The results obtained indicated that zopiclone exists at least as an anhydrate (form A) and a dehydrate (form B). During solubility and dissolution measurements, the anhydrous powders changed into dehydrated zopiclone (figure 16) and no significant difference in aqueous solubility could be detected. Results suggested that the zopiclone dehydrate was less soluble compared to the anhydrous crystal form (Terblanche *et al.*, 2000).

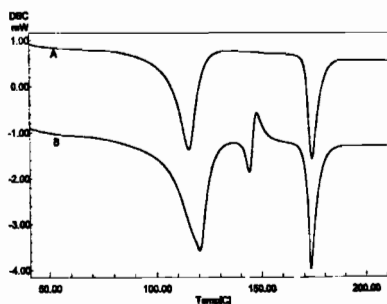


Figure 16 DSC thermograms of zopiclone polymorph A and form B after solubility determinations (Terblanche *et al.*, 2000).

## Roxithromycin

Roxithromycin, a 14-membered-ring, macrolide antibiotic, is an ether oxime derivative of the naturally occurring, macrolide, antibacterial drug, erythromycin (Jarukamjorn *et al.*, 1998). This medically important antibiotic is composed of an erythronolide ring (polyfunctionalised, 14-membered, lactone ring) substituted with desosamine and cladinose sugar units (Gharbi-Benarous *et al.*, 1991).

In order to identify and classify the various crystal forms of roxithromycin, various recommended analytical techniques were used, i.e. XRPD, IR, DSC, TGA and TM, of which XRPD was the primary tool of characterisation. The study was performed on roxithromycin crystals that were recrystallised from various organic solvents. After these characterisation techniques were applied, the physico-chemical properties of the respective crystals were determined. The results from these studies indicated that roxithromycin indeed possessed the ability to crystallise in different polymorphic, pseudopolymorphic and amorphous forms. Six different forms were successfully identified:

1. Form A: Stable, high melting crystal form.
2. Form B: A low melting amorphous forms.
3. Form C: Stable, mid-melting crystal form.
4. Form D: Amorphous, chloroform-solvated form.
5. Form E: A mixture of two crystal forms - likely a low melting point, Form E<sub>L</sub> (95 ±4°C) form and a high melting point, Form E<sub>H</sub> (111°C).
6. Form F: Low melting point Form F<sub>L</sub> transformed into a mid-melting point Form F<sub>M</sub>, which transformed into a high melting point crystal form, Form F<sub>H</sub> (Du Plessis, 2004).

Differential scanning calorimetry is also an unambiguous method of characterisation. Figure 17 is an overlay of the thermograms of roxithromycin polymorphic, pseudopolymorphic and amorphous forms, which showed the differences in melting points between the different forms of roxithromycin (Du Plessis, 2004).

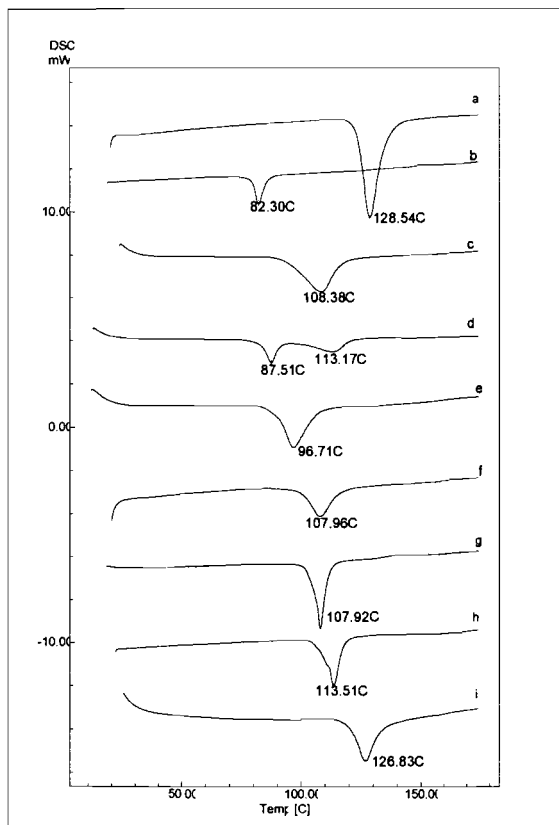


Figure 17 Superimposed thermograms of the different forms of roxithromycin. a) Form A, b) Form B, c) Form C, d) Form D, e) Form E<sub>L</sub>, f) Form E<sub>H</sub>, g) Form F<sub>L</sub>, h) Form F<sub>M</sub> and i) Form F<sub>H</sub> (Du Plessis, 2004).



The thermogram of Form A shows a single peak with a high melting point of 128.97°C, compared to that of Form B, illustrating a single low melting point (82.3 °C). The thermogram of Form C shows the mid-melting point of Form C at 108.38°C. From dichloromethane as recrystallisation solvent it seemed that two forms were obtained, a low (95±4°C) and a high melting point (108±4°C) form. These two forms were referred to as Forms E<sub>L</sub> and E<sub>H</sub> respectively, according to their melting points. According to the heat of fusion rule, it was determined that Forms E<sub>L</sub> and E<sub>H</sub> were an enantiotropic system. Form F crystals appeared to transform from a Form F<sub>L</sub> (107.92°C) into a mid-melting point form F<sub>M</sub> (113.51°C), which further transformed into a high melting point Form F<sub>H</sub> (126.83°C). The transformation was investigated by means of a temperature study involving exposure to increased temperatures in an incubator within a range of 25°C to 115°C. According to the heat of fusion rule, forms are monotropically related when the higher melting point form has the higher heat of fusion, which was the case for the forms of Form F. Although Form C (108.38°C), Form E<sub>H</sub> (108.0°C) and Form F<sub>L</sub> (107.92) had similar melting points, their XRPD diffractograms showed significant differences, indicating three different polymorphic forms (Du Plessis, 2004).

## **Clarithromycin**

Clarithromycin, a derivative of erythromycin, is a 14-membered ring macrolide antibiotic. Its spectrum of activity and clinical uses are very similar to those of erythromycin, but its absorption is more consistent and it has a longer half-life (Dollery, 1999:C248).

In this study, clarithromycin raw material was recrystallised from a number of solvents, and categorised according to the forms already described in literature. Another aim of this study was to prepare and characterise novel polymorphic forms. Two new forms were prepared, i.e. a new polymorphic form from ethyl acetate, Form V, and a chloroform solvate, Form VI. This form showed a melting point of 230°C, somewhat higher than that of the other forms. The existence of solvates was also confirmed with thermal microscopy (figure 18). The DSC and TGA results confirmed the presence of a solvate (De Jager, 2005).

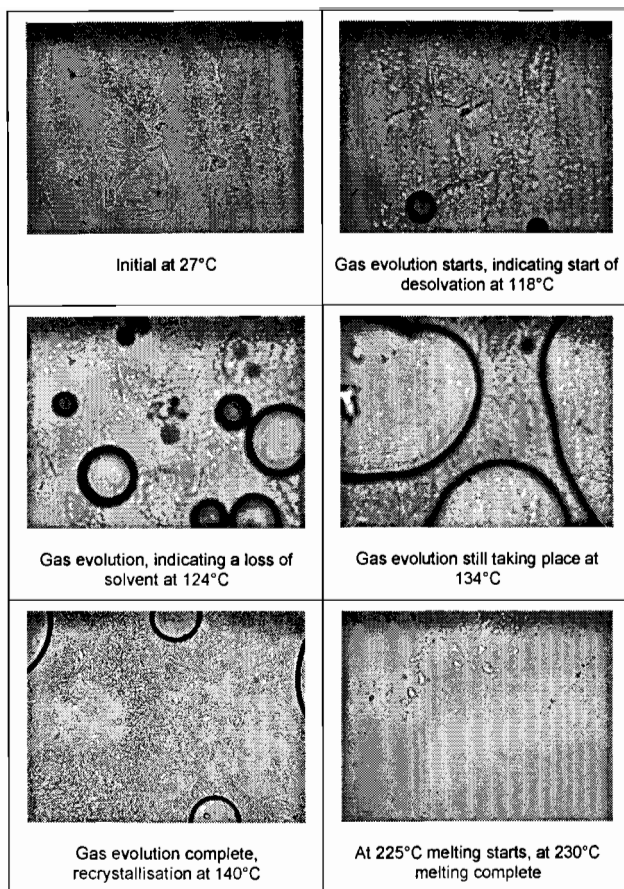


Figure 18 The photomicrographs of clarithromycin chloroform solvate with heating over a temperature range of 27-230°C (De Jager, 2005).

## Conclusion

The physico-chemical stability of an API is an important issue to consider, especially during preformulation, but also during manufacturing. The effects of pharmaceutical processing activities on the crystalline state of polymorphic and solvate systems are important to the pharmaceutical industry. Unanticipated polymorphic changes could lead to unstable or ineffective dosage forms being released onto the market, as well as manufacturing problems, with possibly high cost implications. There currently is no substitute for the proven multidisciplinary studies, of which their goal is to determine the existence of polymorphic forms and/or polymorphic transformations at any time during the handling of active pharmaceutical ingredients.

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