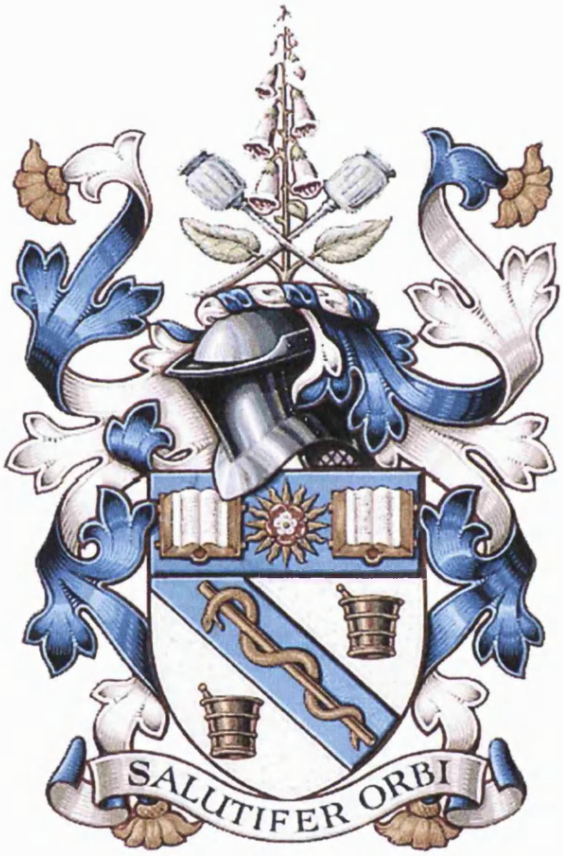
The Use of a Second Excipient to Alter the Characteristics of PVP-Drug Solid Dispersions



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A Thesis Submitted in Partial Fulfilment of the Requirements for the Degree of Doctor of Philosophy

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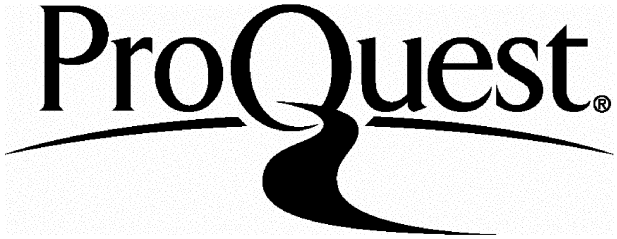

Abstract

Many drugs are poorly water soluble and this can limit bioavailability. One way of increasing the dissolution rate and apparent saturation solubility of a poorly water soluble drug is to prepare a solid dispersion. The ideal solid dispersion is one in which the drug and excipient (typically a polymer) are molecularly dispersed. This dispersion may be unstable. Phase separation and recrystallisation of the drug may occur. The aims of this work were to investigate how the use of a second excipient affects the dissolution of a stable amorphous PVP-drug dispersion, how the use of a second excipient affects the amorphous stability of unstable PVP-dispersions and whether near infrared spectroscopy (NIRS) could be used as a technique to detect crystallisation.

Indomethacin was spray-dried with PVP and a variety of second excipients. Characterisation was performed using differential scanning calorimetry (DSC), X-ray powder diffraction (XRPD), thermogravimetric analysis (TGA) and dissolution studies at pH 1.2, 6.5 and 7.0. At pH 6.5, indomethacin dissolved from all the solid dispersions bar one containing Eudragit S. This dispersion proved to be soluble above pH 7.0. When the dispersion was prepared by dissolving the components of the dispersion in a different order prior to spray drying the dispersion was then found to dissolve suggesting that differences in non-covalent bonding were occurring.

Griseofulvin, flavanone and clotrimazole were all spray-dried with PVP and a variety of second excipients. Characterisation was performed using DSC, XRPD and TGA. The addition of poly(2-hydroxypropylmethacrylate) (PHPMA) was found to improve the amorphous stability of griseofulvin dispersions when stored at 0% and 30% relative humidity (RH) though this was later found to be dependent on the order in which components were dissolved. PHPMA was also found to improve the amorphous stability of flavanone when stored at 0% RH. Sucrose was found to improve the amorphous stability of clotrimazole when stored at room temperature, 0% RH. When dispersions containing lower concentrations of clotrimazole were prepared this benefit was lost although the addition of poly(acrylic acid) to the dispersion did seem to improve the amorphous stability. NIRS investigations of the amorphous stability of griseofulvin dispersions showed that the technique was less sensitive than XRPD, though it may provide some information about the mechanism of stabilisation.

Table of Contents

Acknowledgements

There are so many people to thank along the way;

My supervisors Graham & Steve, I can't thank them enough.

Keef, John & Lionel.

Rachael & Maria.

Nic Wilson for her aid in NIR.

Dave Macarthy for the SEMs.

The EPSRC who funded this work.

Abi, Emma, Laurent, Richard, Matt, Amina, Tony & Elisa, Ray, Mark, Mike P., Brij & Ash, Ketan, Clair, Stefan, Ameet, Viraj, Rita, John, Rosemary, Jean-Luc.

"The rapid progress true Science now makes, occasions my regretting sometimes that I was born too soon. It is impossible to imagine the height to which may be carried, in a thousand years, the power of man over matter. We may perhaps learn to deprive large masses of gravity, and give them absolute levity, for the sake of easy transport. Agriculture may diminish its labour and double its produce; all diseases may by sure means be prevented or cured, not excepting even that of old age, and our lives lengthened at pleasure even beyond the antediluvian standard."

Extract from a letter by Benjamin Franklin to Joseph Priestley (Feb 1780)

"Sapere aude! Dare to know! That is the motto of Enlightenment"

Immanuel Kant (Sept 1784)

Abbreviations

% v/v	Percentage volume in volume
% w/v	Percentage weight in volume
% w/w	Percentage weight in weight
ΔCp	Change in heat capacity
ΔH	Change in enthalpy
CDD	Coherent diffraction domain
DSC	Differential scanning calorimetry
DVS	Dynamic vapour sorption
Ed(s)	Editor(s)
FTIR	Fourier transform infrared
GIT	Gastro-intestinal tract
h	Hours
IR	Infrared
min	Minutes
NIR(S)	Near infrared (spectroscopy)
NMR	Nuclear magnetic resonance
NSAIDs	Non-steroidal anti-inflammatory drugs
PAA	Poly(acrylic acid)
PC	Principal component
PCA	Principal component analysis
PHPMA	Poly(2-hydroxypropylmethacrylate)
PVP	Polyvinylpyrrolidone also known as povidone
PVP-VA	Polyvinylpyrrolidone-vinyl acetate
SEM	Scanning electron microscopy
SNV	Standard normal variant
TGA	Thermogravimetric analysis
T_g	Glass transition temperature
T_K	Kauzmann temperature
T_m	Melting temperature
T_{wb}	Wet bulb temperature
UV	Ultraviolet
XRPD	X-ray powder diffraction

List of Figures

List of Tables

Chapter 1

Introduction

1.1 PREFACE

It is estimated that approximately 40-60% of drugs currently in the development phase are poorly water soluble (Lipinski, 2001; Lipinski, 2002). Poor solubility often limits bioavailability (Leuner and Dressman, 2000). The use of a solid dispersion, in which the drug is combined with one or more inert carriers on the nano or molecular scale is one way in which the dissolution issue can be overcome (Serajuddin, 1999). This thesis concerns the development of solid dispersions comprised of a hydrophobic drug and two excipients. Several excipients were examined, all but one being polymers. Selection criteria were based on a hypothesis that directed non-covalent interactions between excipients and drug could be used to increase the stability of a dispersion. This introduction will describe what polymers are and how they are used, the conditions in the gastro-intestinal tract and the methods used to increasing the solubility of poorly water soluble drugs, including the use of solid dispersions.

1.2. POLYMERS IN PHARMACEUTICAL PRODUCTS

According to The New Oxford Dictionary of English, the term polymer originated in Germany in the mid 19th Century with the definition "having many parts". It was derived from the Greek *polus* meaning many or much and *meros* meaning a share. Whilst naturally occurring polymers such as rubber or silk have been used for many years, polymer chemistry is a relatively young branch of science. This science only gained widespread acceptance in the late 1920s and did not really blossom until the later half of the 20th Century, despite the fact that Leo Baekeland produced the first synthetic polymer in 1907. Dr H. Staudinger (later a Nobel Prize winner) first proposed the theory of large molecular weight molecules in 1920. However, it was not until Dr Herman Mark presented his X-ray crystallography studies of cellulose in Germany in 1926 that something approaching true proof was found.

From the early 1930s through to the 1940s a number of synthetic polymers were developed and marketed. These included Nylon, Perspex or poly(methyl methacrylate), polyvinylchloride (which was originally observed in the 1870), and a range of silicone-based polymers and epoxy resins. In the aftermath of the Second World War there was a realisation that one could not rely solely on natural polymers and their precarious trade routes and it was this that provided an impetus for the development of synthetic polymers that could be used as replacements.

There are many uses for synthetic polymers and of these a large number are as replacements for more traditional materials such as wood, textiles, natural rubber or metals. Synthetic polymers with biomedical uses are a more recent development that began during the 1970s. These biomedical polymers have a wide range of uses beyond being solely used as bulking agents. Example of other uses include; altering drug release characteristics (Li *et al.*, 2005), which may result in pseudo-zero order release (Ranade, 1991), protection of the gastric mucosa from drugs implicated in the development of gastric and duodenal ulceration (Lanza *et al.,* 1980), colonic drug delivery (Van den Mooter, 2006), or being covalently bonded to a drug molecule to alter its pharmacokinetics (Taylor, 1996). Whilst there are a large number of products which use polymer coats to provide altered release profiles, they may also be used to create solid dispersions.

1.3 PHYSIOLOGY OF THE GASTRO-INTESTINAL TRACT (GIT)

On swallowing, food passes down the 25 cm of the oesophagus, through the gastro-oesophageal sphincter and into the stomach. The stomach is approximately 25 cm long but its volume varies depending on the amount of food it contains. An empty stomach typically has a volume of 50 ml yet when distended this volume can reach as much as 4 L. The smooth lining of the stomach is dotted with millions of deep gastric pits that lead into the gastric glands that collectively produce gastric juice. The main components of gastric juice are water, Hydrochloric Acid (HCl), pepsin and intrinsic factor. HCl makes the stomach contents extremely acidic (Table 1.1). This helps kill many of the bacteria present in food and is necessary for the activation and optimal activity of pepsin. Pepsin is secreted as the inactive pepsinogen. It is an enzyme that breaks down proteins. Intrinsic factor is a glycoprotein required for the absorption of vitamin B_{12} in the small intestine.

The stomach mucosa is exposed to some of the harshest conditions in the entire digestive tract; a high level of acidity and the presence of pepsin which can digest the stomach itself. To protect itself, the stomach secretes a thick alkaline mucus that builds up on the stomach wall. Therefore, whilst the stomach has a relatively large epithelial surface absorption is limited due to this thick mucous layer. The epithelial cells of the mucosa are joined together by tight junctions that prevent gastric juice from leaking into the underlying tissue layers. Damaged epithelial cells are shed and quickly replaced, with the whole of the stomach epithelium being replaced every 3-6 days.

Region	pH
Stomach	1.0-2.5
Small intestine	
• duodenum	5.5-6.0
• jejunum	6.0-7.0
• ileum	7.0-7.5
Large intestine	
• caecum	6.4-7.0
• colon	7.0-7.5

Table 1.1 pH values for the gastro-intestinal tract.

From the stomach the partially digested food passes into the small intestine. The duodenum, defined as the first 20-30 cm of the small intestine following on from the stomach, has a thick wall with a deeply folded mucous membrane and contains duodenal digestive glands and Brunner's glands. The Brunner's glands produce a protective alkaline secretion, free from enzymes, which neutralises the HCl found in gastric fluid. This causes a sudden change in the pH of chyme (the food/gastric fluid matter) entering the small intestine (Table 1.1). The jejunum and the ileum follow on from the duodenum and make up the rest of the small intestine. There is no absolute demarcation between them, although the jejunum has a thicker wall than either the ileum or duodenum. In total the small intestine is around 2 m long. The small intestine is highly adapted for nutrient absorption. Not only is it long, it has three main structural features which increase surface area. The plicae circulares are large, circular folds that increase the surface area. Villi are finger-like projections that increase surface area. Microvilli are tiny projections found on the surface of the cells of the villi and these further increase the surface area over which absorption can occur.

Whilst gastric emptying varies quite wildly with the amount of food digested (Davis *et al.*, 1988) and with the type of formulation (Christensen *et al.*, 1985), the transit time along the small intestine is considered to be relatively stable. It has been suggested that it takes around 3 h (± 1 h) for a dosage form to reach the large intestine (Davis *et al.*, 1986), though another research group has suggested that the mean small intestine transit

time for a radiotelemetry capsule was 5.7 h with a standard deviation of 2.0 h (Evans *et al.*, 1988).

The large intestine has a greater diameter than the small intestine but at around 1.5 m long it is actually shorter. As most food digestion and absorption occurs in the small intestine there is little of either process occurring in the large intestine. Water, electrolytes and vitamins produced by the bacterial fauna present in the large intestine are to some degree absorbed over the 12 to 24 h that faecal matter spends in the large intestine.

1.4 INCREASING SOLUBILITY AND DISSOLUTION RATE

As can be seen in the modified Noyes-Whitney equation (Equation 1.1) the following parameters can be altered to improve the dissolution rate of a particle; increasing the surface area available for dissolution by decreasing particle size and/or increasing wettability, decreasing the boundary surface thickness, ensuring sink conditions for dissolution and increasing the apparent saturation solubility (Noyes and Whitney, 1897; Nernst, 1904). Sink conditions in the GIT whilst linked to the apparent saturation solubility are essentially uncontrollable and the volume and content of the gastrointestinal fluids will both have large effects (Leuner and Dressman, 2000). Various systems have been utilised to increase solubility by altering the other parameters, with salt formation, solubilisation and particle size reduction (micronisation) being the most common (Wadke and Serajuddin, 1989). These and other techniques will be discussed below.

$$dC/dt = (AD[C_s - C])/h$$

Equation 1.1 Modified Noyes-Whitney equation

Where:

dC/dt = rate of dissolution

A = surface area available for dissolution

D = diffusion coefficient of the compound

C_s = solubility of the compound in the dissolution media

C = concentration of compound in the media at time t

h = thickness of diffusion boundary

1.5 CHEMICAL MODIFICATION

A drug can be chemically modified to produce a more soluble prodrug (Albert, 1958), where the term prodrug is defined as "a bio-reversible chemical derivative of an active parent drug" (Taylor, 1996). In its strictest sense this can include salt forms and complexes that are designed to easily disassociate from the drug (Anderson, 1985). For example, diclofenac is typically formulated as a sodium salt, however Voltarol Rapid® is a formulation containing the potassium salt and as its name suggests it dissolves more rapidly owing to the increased disassociation of the potassium salt. Regardless of the mechanism of modification the aim is to induce a higher solubility by reducing the lattice enthalpy of a drug or by introducing an ionisable group (Amidon, 1981).

Salt formation, by its nature requires a drug to be basic or acidic; therefore it is unsuitable for neutral drugs such as griseofulvin (Berge *et al.*, 1977). It may prove to be unfeasible or uneconomic to create a salt form of a particular drug using salts that are non-toxic or accepted as safe by the various regulatory bodies. There is also a risk that a salt form of a drug will aggregate in the GIT, thus removing any dissolution advantage that had been hoped to be gained (Serajuddin, 1999).

The use of a progroup to increase solubility can produce a number of issues. In an ideal situation the prodrug in solution would be degraded to drug and progroup by membrane-bound brush boarder enzymes to release the drug and progroup. If this does not occur, passive absorption (which is dependent on the lipophilicity of the drug) may be limited by the hydrophilic progroup (Fleisher *et al.*, 1996). The use of a prodrug requires an understanding of the kinetics and mechanisms of prodrug degradation, as a number of drugs, including griseofulvin have been altered to display increased solubility, which has not resulted in bioavailability enhancements despite being known to degrade at the intestinal lumen. It was suggested that incomplete conversion, concurrent metabolism to inactive derivatives or elimination from the body before conversion was complete was to blame. The production of digoxin prodrugs and complexes with hydroquinone, in an attempt to reduce the crystal lattice enthalpy has proved to be successful and indeed a digoxin prodrug product is available in the US (Acylanid®), however the actual advantage is debatable as the original product works well (Higuchi and Ikeda, 1974; Stella, 1975).

1.6 MICRONISATION

Micronisation and other particle size reduction techniques have been successfully used to increase the dissolution rate and apparent equilibrium solubility of a number of drugs. As predicted by the modified Noyes-Whitney equation (Equation 1.1), particle size correlates well with dissolution rate (Maillols *et al.*, 1982). Furthermore, the surface area over which drug dissolution occurs can be increased by increasing particle roughness (Mosharraf and Nystrom, 1995).

The therapeutic dose of griseofulvin has been reduced by 50% through micronisation (Atkinson *et al.*, 1962; Kabasakalian *et al.*, 1970). Ultra-micronised dispersions of griseofulvin have been shown to display further improvements in bioavailability (Straughn *et al.*, 1980). It has been stated that poor wettability and handling difficulties are an issue with very small particles (Serajuddin, 1999). Increased surface energy causing increased van der Waal's forces raises the possibility of agglomeration and aggregation that may cause a net decrease in dissolution. This has been shown *in vitro* with griseofulvin and glutathione (Lin *et al.*, 1968), although another group demonstrated the reverse with griseofulvin (Chiou and Riegelman, 1969b; Chiou and Riegelman, 1971b).

1.7 SOLUBILISATION

Solubilisation is the use of an inert molecule to improve the solubility of a drug molecule. There are many ways in which the solubility of a drug may be increased. Cyclodextrins are amphoteric molecules that complex with one or more drug molecules and on dissolution this complex should separate. Unfortunately cyclodextrins have a tendency to aggregate in solution which limits their usefulness (Loftsson and Brewster, 1996; Loftsson and Másson, 2004; Rajewski and Stella, 1996). Micelles which self assemble in solution from amphiphilic block co-polymers have a hydrophilic exterior and a hydrophobic interior can also solubilise poorly water soluble drugs (Kwon, 2003; Kataoka *et al.*, 2001).

1.8 THE CRYSTALLINE AND AMORPHOUS FORMS

A solid crystal can be defined as a mass of ions, atoms or molecules, which are packed in a neat, rigid, fixed and orderly fashion (the crystal lattice). This gives both long and short range order. The basic building block of a particular crystal is named the unit cell.

It is repetition of this cell in three dimensions that produces a crystal (Florence and Attwood, 1998; Yu, 2001; Mullin, 2001). Crystalline solids have defined thermodynamic properties such as solubility and melting point. The phenomenon of polymorphism, where molecules arrange themselves into a different unit cell, and solvate forms, where solvate molecules such as water (specifically called a hydrate) or ethanol make up part of the crystal lattice (pseudo-polymorphism) mean that a particular molecule can exist in a number of different, but definable states, with different physical and chemical properties (Rustichelli *et al.*, 2000; Brittain, 2002). Aside from these ordered states a molecule can exist in a disordered state called the amorphous form. Whilst amorphous materials may exhibit short range order, they lack long range order (Craig *et al.*, 1999).

The major advantages that the amorphous form and metastable polymorphs (when these have a reduced crystal lattice enthalpy) have to offer over a stable crystalline form are an increase in apparent saturation solubility and an increase in dissolution rate (Hancock and Parks, 2000; Shekunov and York, 2000). This is due to a reduction in the strength of inter-molecular bonding which results in a decrease in the amount of energy required to dissolve a drug. Calorimetric investigations of the amorphous form have been conducted on a wide range of drugs. These suggested that a 10 to 1600-fold increase in solubility of the amorphous form versus the crystalline form might be achieved. It is very important to note that the observed solubility advantage was usually considerably lower, but was considered significant (Hancock and Parks, 2000).

The wettability of a drug particle and therefore its dissolution rate can also be increased by merely introducing disorder into the surface of a particle. Nevertheless this has been shown to be an unstable improvement (Elamin *et al.*, 1994). Surface disorder has also been shown to increase the apparent equilibrium solubility (Mosharraf and Nystrom, 2003).

1.8.1 FORMATION OF AMORPHOUS SOLIDS

Amorphous character is generally considered to be the preserve of polymeric materials; even so it can be induced in smaller molecules, either by accident or on purpose. There are four main methods by which this can be done; condensation from the vapour state, super-cooling of molten material, milling or other mechanical manipulations creating disorder and rapid precipitation from solution, which includes spray drying. In the

literature the production of amorphous content is most commonly described by the process of super-cooling (Hancock and Zografi, 1997; Craig *et al.*, 1999).

Normally, as a liquid is cooled, there is a steady decrease in enthalpy and volume until the liquid reaches its melting temperature (T_m) where crystallisation occurs. At this point there is a sudden decrease in both specific volume (V) and enthalpy (H) that results from the orderly packing of molecules into a crystal lattice (Figure 1.1). Should a liquid be super-cooled (say by being poured into liquid nitrogen), the molecules are not given enough time to orientate themselves into a lattice and consequently there is no sudden decrease in volume or enthalpy. This produces a "super-cooled liquid" or "rubbery state". The plot of temperature against volume or enthalpy continues along the same slope as the equilibrium line for the liquid until a critical temperature, the glass transition temperature (T_g) is reached. At this point there is a deviation from the equilibrium line giving rise to the glassy state which has a higher enthalpy and volume than would be expected from the super cooled liquid.

Theoretically, one could extrapolate the thermodynamic properties of the liquid and super-cooled liquid state beyond the glass transition. However this gives rise to the Kauzmann paradox wherein a super-cooled liquid would have a lower energy, entropy and volume than the crystalline and this would be a violation of the third law of thermodynamics. The temperature at which this extrapolated line and the line relating to the crystalline substance meet is named the Kauzmann temperature (T_K) and this is thought to represent the theoretical lower limit of T_g, as the excess entropy of the super-cooled liquid would be the same as that of the crystal (Kauzmann, 1948; Stillinger, 1988; Stillinger *et al.*, 2001).

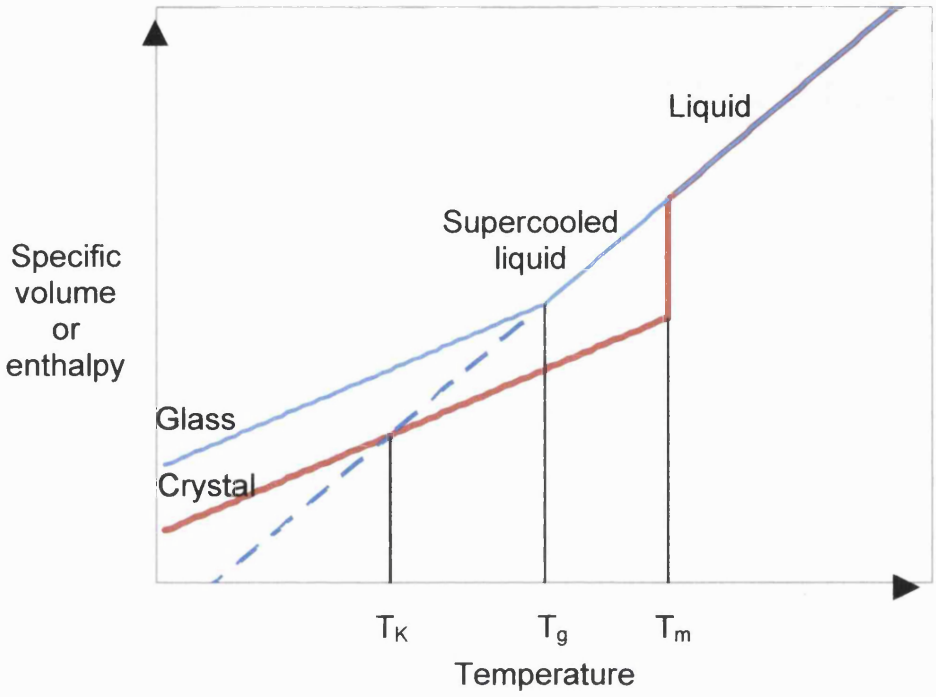

Figure 1.1 Graph depicting the effect of temperature on specific volume and enthalpy. Adapted from Hancock and Zografi (1997).

1.8.2 THE CRYSTALLISATION PROCESS

Crystallisation is most commonly described from a solution, though it can occur spontaneously from the amorphous state. The production of a supersaturated solution, i.e. the state in which the liquid (solvent) contains more dissolved solids (solute) than can ordinarily be accommodated at a particular temperature is generally considered to be the first stage in inducing crystallisation.

The formation of crystal nuclei (nucleation) can be split into two main forms. Primary crystallisation occurs when there is a spontaneous coming together of a sufficient quantity of solute molecules that they do not redissolve. Secondary crystallisation is the initiation of crystallisation by the addition of crystals of solute. The active addition of crystals is called seeding. Impurities such as dust particles or the presence of solid objects (such as a stirrer or the wall of a vessel) may also act as seeds for crystallisation. Whether this is grouped with primary or secondary crystallisation is debatable, owing to the fact that experimentally it is difficult to distinguish between crystallisation caused by impurities or solid objects and spontaneous crystallisation (Mullin, 2001).

It is possible to create different polymorphic forms during the crystallisation process by altering the type of solvent, which alters the way in which the solvent and solute interact. It is also possible to create different polymorphic forms by changing the temperature at which crystallisation is performed or by altering the rate at which supersaturation is achieved (Mullin 2001).

The area of crystal growth is the subject of a number of theories (Mullin 2001). These theories are discussed below:

Diffusion Theory: This can be thought of as a reversal of dissolution and as such would be governed by the modified Noyes-Whitney equation (Equation 1.1). In this model cooling rate is important as a faster cooling rate will result in smaller more numerous crystals whilst a slower rate will produce larger crystals as the time frame over which crystal growth can occur is larger.

Surface energy theory: This theory compares the growing crystal to an isolated liquid droplet. A droplet assumes the minimum surface area resulting in a minimum surface free energy, as this is favoured thermodynamically. Similarly, the growing crystal would always tend towards the shape giving the lowest free energy for a given volume. There is little evidence to support this theory and the theory does not encompass the effect of varying supersaturation and solution movement on crystal growth.

Absorption-layer theory: Volmer first proposed absorption-layer theory in 1939 though it has been modified since. The theory relates crystal growth to thermodynamic reasoning; solute molecules or similar coming into contact with the crystal surface are not immediately incorporated into the lattice and are instead free to migrate across the surface producing an interface between the solution and the crystal. These building blocks will therefore migrate to the position in the lattice where attractive forces are most strong. This will continue until the face is completed. Further layers are then added, though a "centre of crystallisation" must be created, a process also called surface nucleation.

1.8.3 THE STABILITY OF AMORPHOUS SUBSTANCES

The amorphous form is generally considered to be unstable and disorder introduced during the production of a pharmaceutical may be lost on storage (Lane and Buckton,

2000). It is generally thought that amorphous substances are highly unstable above their T_g owing to increased molecular mobility, and more stable below their T_g, when reduced molecular mobility within the system prevents destabilisation of the disordered form due to its higher viscosity. However, this general concept is not clear-cut. Molecular motion in amorphous products below their T_g has been observed (Angell, 1995). Recrystallisation is therefore possible over longer time periods (Duddu *et al.*, 1997).

Hancock *et al.* (1995) described the molecular mobility of amorphous solids below their T_g. They measured the molecular mobility of three substances commonly used in pharmaceutical formulations; indomethacin, polyvinylpyrrolidone (PVP) and sucrose. They did this at various temperatures below their known T_g and after various time periods. By the use of Differential scanning calorimetry (DSC) these researchers were able to conclude that storage at a temperature at least 50 °C below the T_g of each substance was required in order to be certain that the molecular motions detected would be negligible over the shelf life of the products. Similar research has been carried out to investigate the stability of amorphous substances at varying storage temperatures with respect to T_g (e.g. Elamin *et al.*, 1994; Duddu *et al.*, 1997; Hatley, 1997). Such research aids the accurate prediction of the shelf life of an amorphous product at given temperatures/water contents (e.g. Duddu and Dal Monte, 1997; Hatley, 1997).

1.9 SOLID DISPERSIONS

1.9.1 HISTORICAL OUTLINE

The production of a solid dispersion may be used to increase the apparent saturation solubility and dissolution rate of a drug molecule. Whilst this may result in the production of amorphous material this is not always an immediate goal. Sekiguchi and Obi first mooted the idea of a solid dispersion in 1961. They proposed the creation of a eutectic mixture (the definition of which is the combination of two solids that displays the most depressed melting point, most commonly used in the science of metal alloys) of drug dispersed in a physiological inert carrier with a good solubility profile. They demonstrated increased dissolution and absorption of sulfathiazole in man when urea was used as a carrier (Sekiguchi and Obi, 1961). Later they examined the release of chloramphenicol from urea and the absorption in rabbits and showed an improvement in absorption when compared with the regular formulation (Sekiguchi *et al.*, 1964). It has also been demonstrated that eutectic mixture display more rapid dissolution than non-

eutectic dispersions (Chiou and Riegelmann 1971b; Ford, 1984; Ford and Rubinstein, 1978). Two early papers were published that suggested that an increase in the dissolution rate of a drug from a solid dispersion would be seen if the drug was molecularly dispersed to produce, in effect, a "solid solution" (Levy, 1963; Kanig, 1964). The advantages of a solid dispersion or solution over a conventional system are shown in Figure 1.2.

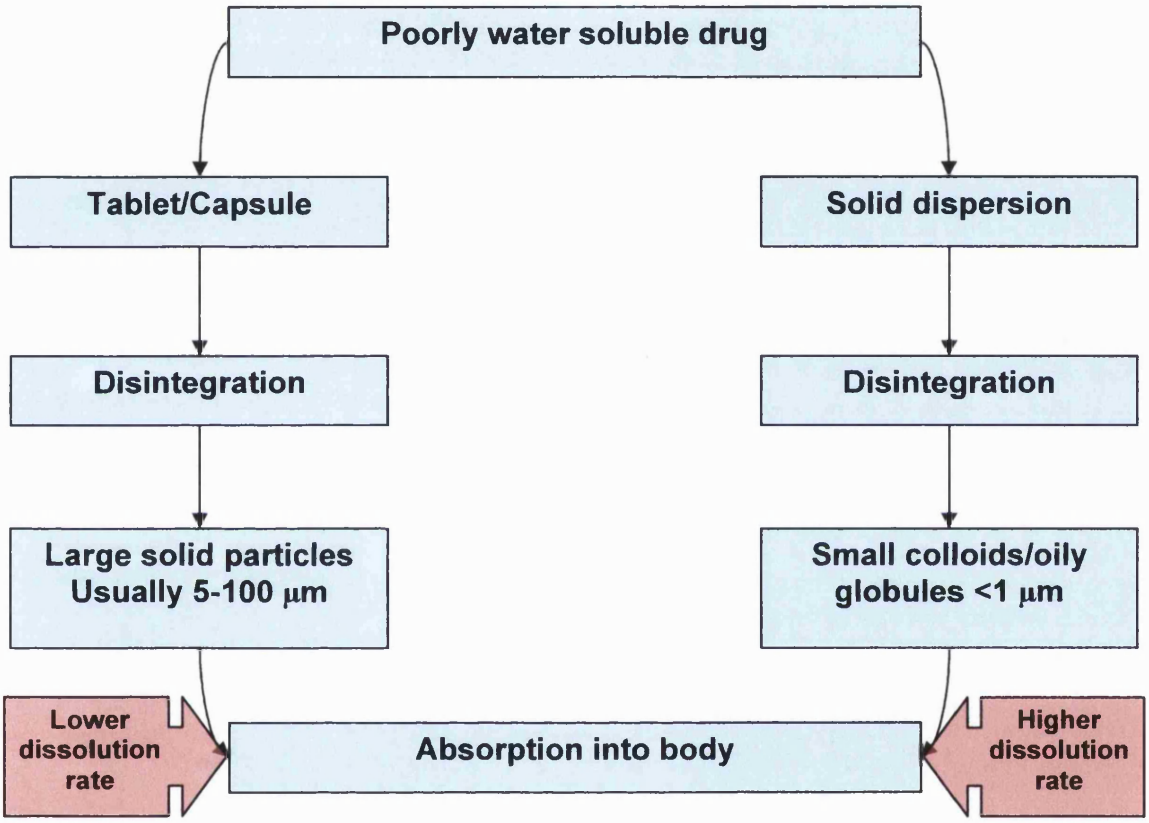

Figure 1.2 The dissolution of a poorly water soluble drug from a solid dispersion compared with the dissolution from a more traditional dosage form showing the possible bioavailability enhancement resulting from reducing particle size. Adapted from Serajuddin (1999).

Early experiments involving solid dispersions tested a range of low molecular weight carriers and poorly water soluble drug systems such as urea with paracetamol (Goldberg *et al.*, 1966a) or with chloramphenicol (Goldberg *et al.*, 1966c) and succinic acid with griseofulvin (Goldberg *et al.*, 1966b). All of these showed improved dissolution or absorption characteristics or both. Tachibana and Nakamura (1965) first described the use of the polymer, PVP, to create dispersions of β-carotene using a solvent evaporation technique. Mayersohn and Gibaldi (1966) were one of the first groups to demonstrate

the feasibility of the use of PVP dispersions to increase dissolution using the drug griseofulvin though they conceded it was possibly a solvate of the drug. Griseofulvin has also been dispersed in PEG 6000 and this product displayed fast dissolution and almost complete absorption in dogs. This compared favourably with the 30-60% griseofulvin absorption from commercial products and the 40% griseofulvin absorption from micronised material. Later the bioavailability benefits of solid dispersions were demonstrated in man (Chiou and Riegelman, 1969a; Chiou and Riegelman, 1969b; Chiou and Riegelman, 1970; Chiou and Riegelman, 1971a). These effects were not seen when physical mixes of griseofulvin and PEG were used (Chiou and Riegelman, 1971b).

Beyond the aforementioned studies by Chiou and Riegelman, griseofulvin also showed greater bioavailability when formulated with poloxamers (Heyer and Fromming, 1983). Other drugs that have shown bioavailability improvements include dicumarol-PVP (Sekikawa *et al.*, 1983), mebendazole-PEG 6000 dispersions (Chiba *et al.*, 1991), triamterene-D-mannitol dispersions (Arias *et al.*, 1994) and triamterene-PEG 6000 dispersions (Arias *et al.*, 1996).

Ultimately, whilst more than 500 papers have been published on the subject and various materials are employed as drug carriers (Leuner and Dressman, 2000), only two commercial products, a griseofulvin in polyethylene glycol 8000 solid dispersion (Gris-PEG, Novartis) and a nabilone in PVP solid dispersion (Cesamet, Lilly), have been marketed during the last four decades following the initial work of Sekiguchi and Obi (1961). This lack of commercial realisation is largely due to physical and chemical instability or scale-up problems (Franco *et al.*, 2001; Serajuddin, 1999; Craig, 2002).

1.9.2 THE STABILITY OF SOLID DISPERSIONS

Like any pharmaceutical product a solid dispersion should prove to be stable across the shelf life of the product. Its dissolution profile should not change on storage, nor should any other changes occur. One of the earliest stability studies was performed using indomethacin-PEG 6000 dispersions. On storage at temperatures between 25 °C and 45 °C at 71% RH, dissolution was shown to steadily decrease before displaying a slight increase. This was ascribed to crystallisation of the indomethacin as concurrent to this dissolution decrease the tablets became less yellow with time (amorphous indomethacin is yellow, crystalline indomethacin is off-white) (Ford and Rubinstein, 1980). Even a

colour change such as this would be cause for concern in a licensed pharmaceutical product. Both decreases and increases in dissolution rate have also been observed for PEG dispersions of temazepam and triamterene (Dordunoo *et al.*, 1997).

With regard to PVP, the general aim is most often to prolong the stability of amorphous drug character. PVP has been shown to be an excellent candidate for this. There is some debate over the mechanism by which this stabilisation occurs. Interactions with PVP have been seen in a number of drugs and on occasion the researchers involved will state that interactions are more important than T_g when considering recrystallisation, or formation of crystals during processing. One of the earliest studies described in the literature to consider interactions as a driving force for stabilisation did not actually investigate interactions directly. Instead the development of melt endotherms on storage was investigated. While cholesterol was not stabilised by PVP and griseofulvin was only stabilised to a small degree, methyl-p-hydroxybenzoate was fairly well stabilised. When the stability of methyl-p-hydroxybenzoate and methyl-p-aminobenzoate were compared the former was found to be stabilised to a greater degree (Shefter and Cheng, 1980). The interactions considered in this study were between the carbonyl of PVP and the hydroxyl and amino groups of methyl-p-hydroxybenzoate and methyl-p-aminobenzoate.

Indomethacin and PVP dispersions have been investigated for over 20 years. Imaizumi and co-workers conducted the first study to show that indomethacin was stabilised by PVP although they did not discuss mechanisms (Imaizumi *et al.*, 1983). Zografi and co-workers have studied indomethacin crystallisation at some length. They found that the form to which indomethacin crystallised was dependent on the storage temperature. When the indomethacin was stored just below or above the T_g a meta-stable form was seen alongside the stable polymorph. When the amorphous indomethacin was stored below its T_g only the stable polymorph was seen (Yoshioka *et al.*, 1994).

Their first paper on indomethacin stabilisation showed that in order to inhibit crystallisation of pure amorphous indomethacin a storage temperature 40-50 °C below T_g was required. Conversely, similar levels of inhibition in indomethacin and PVP dispersions could be seen at 5 °C above the T_g (Yoshioka *et al.*, 1995). Following this a spectroscopic examination was made and it was concluded that in the pure amorphous form indomethacin exists as diamers. These diamers are connected through the

carboxylic acid group. They form the basis of the stable (γ-) form, though not the meta stable (α-) form. PVP, through the carbonyl group, interacts with the carboxylic acid group of indomethacin, breaking up these diamers thus preventing recrystallisation to the stable form (Taylor and Zografi, 1997; Crowley and Zografi, 2003). Having examined the effect of PVP chain length and substitution of pyrrolidone groups with vinyl acetate groups they came to the conclusion that substitution and chain length were less important than the absolute number of sites of interaction (Matsumoto and Zografi, 1999). One especially interesting paper showed a correlation between the level of interaction between a poorly water soluble drug and PVP and the amount of water that was absorbed by the PVP, which would affect the stability of a sample dispersion (Crowley and Zografi, 2002). Another group compared the apparent equilibrium solubility (also referred to as the apparent saturation solubility) of indomethacin when combined with PVP versus silica with which it did not interact. It was their conclusion that "the apparent equilibrium solubility is affected solely by the state of indomethacin, irrespective of the carrier species, and despite carrier-dependent chemical interactions" (Watanabe *et al.*, 2003).

Accelerated stability studies involving frusemide-PVP dispersions showed these to be relatively stable except when stored at 45 °C, 75% RH. This stability was attributed to an interaction between the sulfonamide group of frusemide and the carbonyl group of PVP (Doherty and York, 1987; Doherty and York, 1989). A similar study using oxodipine-PVP dispersions showed that when stored for 18 months at up to 55% RH recrystallisation did not occur or could not be seen, however at 65% RH, crystallisation did occur (Guillaume *et al.*, 1992). Studies involving piroxicam and PVP have shown a similar interaction through either the hydroxyl or amine group of piroxicam but this was heavily concentration dependent and when PVP K30 was used piroxicam was only found to be fully amorphous up to a concentration of 20% (Tantishaiyakul *et al.*, 1996; Tantishaiyakul *et al.*, 1999). On storage for 12 months at 45 °C and ambient temperature, slight decreases in piroxicam dissolution were seen (Ingkatawornwong *et al.*, 2001) though crystallisation was not seen.

Other drugs and small molecules which have displayed interactions with PVP include clofazimine (Narang and Srivastava, 2002), paracetamol (Afrasiabi Garekani *et al.*, 2003), probucol, which interestingly was found to form a metastable crystal in PAA and PEO in contrast to the amorphous form in PVP (Yagi *et al.*, 1996; Broman *et al.*, 2001),

carbamazepine (Sethia and Squillante, 2004), diflunisal (Rodriguez-Espinosa *et al.*, 1998), phenytoin (Franco *et al.*, 2001) and lactose, where stability was felt to be more dependent on interactions than T_g (Berggren and Alderborn, 2004).

Conversely PVP has been shown to stabilise ketoconazole in the amorphous form without specific interactions. It has been suggested that this is due to an "anti-plasticising" effect, which is in essence, increased viscosity at a molecular level leading to decreased Ostwald ripening or drug migration and lattice formation (Van den Mooter *et al.*, 2000). Drugs that have been formulated with PVP which, whilst showing an increased dissolution rate, were not observed to interact include albendazole, which was also formulated with PEG and again did not display an interaction (Mallick *et al.*, 2003) and flunarizine (Marin *et al.*, 2002).

In a recent paper, seven drugs were investigated for their hydrogen bonding capabilities, four of these contained carboxylic acid groups; BAY 12-9566, naproxen, ketoprofen and indomethacin, whilst one contained hydroxyl groups (testosterone), one an amide (phenacetin) and one contained no proton donating groups (progesterone). Overall it was found that those that interacted with Gelucire 50/13 (a brand of polyglycolised glycerides) did not revert to the crystalline form on storage (Gupta *et al.*, 2002).

A group more interested in the use of film coating produced some interesting results that are highly applicable to the production of dispersions. Whilst their primary interest was the suppression of the T_g of PVP by various drugs, as a by-product they analysed the miscibility of drugs in PVP. Paracetamol, naproxen, salicilamide, carbamazepine and propranolol were all miscible with PVP and displayed hydrogen bonding to the PVP. The T_g of PVP was suppressed and all were found to be amorphous up to 30% w/w. Griseofulvin was also studied and it was found that it did not interact with PVP nor did it prove to be amorphous in their study (Nair *et al.*, 2001).

As well as crystallisation of the active, changes in the physical form of the carrier can also occur and these can affect the dissolution of drugs from solid dispersions. When a PVP dispersion containing an antiviral (UC-781) that interacted with PVP through a secondary amine group was put on a long term stability study at 4-8 °C and 25 °C (25% RH), changes in dissolution rate were seen. The system remained amorphous, suggesting that changes in the polymer were resulting in changes in dissolution (Damian

et al., 2001; Damian *et al.*, 2002). Indomethacin-Eudragit (RS, E and blends of the two) dispersions were investigated for changes in the physical form of the Eudragit on storage. Whilst the T_g was seen to remain constant during storage and the indomethacin was not seen to crystallise, enthalpic relaxation did occur, suggestion that the polymer network was becoming increasingly ordered. The drug diffusion coefficient was seen to alter in indomethacin-Eudragit E dispersions suggesting that there was an interaction between the two (Lovrecich *et al.*, 1996). Particle size changes can also have an effect on dissolution (Chiou and Niazi, 1976).

1.9.3 PRODUCTION OF SOLID DISPERSIONS

In essence there are two main methods of production. The first relies on melting either the carrier or the drug or both. If only one component is melted then the second is dissolved in it. The slag/solution is then cooled to produce a solid dispersion. Sekiguchi and Obi (1961) produced their dispersions by melting eutectic mixtures, which by definition have the lowest melting point though even then the temperatures they used (>110 °C) could cause the constituents to degrade (Chiou and Riegelman, 1971b). Similarly Goldberg and co-workers used temperatures in excess of 100 °C to produce their paracetamol-urea, griseofulvin-succinic acid and chloramphenicol-urea dispersions (Goldberg *et al.*, 1966a; Goldberg *et al.*, 1966b; Goldberg *et al.*, 1966c).

The other main technique is to dissolve the components in a mutual solvent and then evaporate/dry the solvent. This is most commonly performed using rota-evaporation under reduced pressure (Chiou and Riegelman, 1971b). One of the drawbacks of rota-evaporation is that it produces a thin film which must then be scraped free from the vessel. Lyophilisation, which can also be used to produce dispersions, results in a low density mass which must be further processed in order to produce a free flowing powder (Rogers *et al.*, 2002). Advancements have meant that it is now possible to spray dry from organic solvents. Spray drying will be the method used in this thesis.

1.10 POLYMER BLENDS, HYDROGEN BONDING AND THE USE OF BLENDS IN SOLID DISPERSIONS

"The critical value of the interaction free energy is so small for any pair of polymers of high molecular weight that it is permissible to state as a principle of broad generality that two high polymers are mutually compatible with one another if their free energy of interaction is favourable, i.e. negative. Since the mixing of a pair of polymers, like the

mixing of simple liquids, in the great majority of cases is endothermic, incompatibility of chemically dissimilar polymers is observed to be the rule and compatibility is the exception. The principle exceptions occur among pairs in possession of polar substituents which interact favourably with one another."

P.J Flory, Principles of Polymer Chemistry 1953

Blending two polymers is a relatively easy way to combine the desirable properties of different polymers (Tomkins *et al.*, 2005). Blends have been used to produce zero-order release characteristics from sustained release formulations (Gupta *et al.*, 2001; Nerurka r*et al.*, 2005). Semi-solid blends of tetraglycol and PEG 6000 have been used to solubilise glibenclamide for oral release (Galal *et al.*, 2003). The use of polymer blends as scaffolds for tissue engineering or regeneration is currently being investigated as many blends are composed of polymers that have already been shown to be biocompatible such as silk fibroin and chitosan (Gobin *et al.*, 2005), polyvinyl alcohol (PVA) and hydroxyapate (*et al.*, 2004) and poly(lactic-co-glycolic acid) and PVA (Oh *et al.*, 2003). As stability may be related to T_g a good example of a relevant blend of polymers would be that produced by Lau and Mi (2002). They examined the effect of combining PVP with PAA and showed that when together the T_g of the molecular dispersion in a ratio of 50:50 was higher than either of the constituents; 205 °C for the blend against 184 °C for PVP and 106 °C for PAA. This was ascribed to hydrogen bonding which was seen by infrared and solid state NMR. This interaction has been observed previously (Florence and Attwood, 1998).

In Section 1.9.2 a selection of literature was presented that covered some of the interactions that have been observed in solid dispersions. One reason for blending polymers would be take advantage of different functional groups. The miscibility of indomethacin and citric acid has been increased by the addition of PVP to the solid dispersion. In high concentrations of indomethacin and citric acid crystallisation was seen during preparation (Lu and Zografi, 1998). Ibuprofen-PVP-urea and ibuprofen-PVP-PEG ternary systems have been produced and compared with binary systems (Cirri *et al.*, 2004). Six and co-workers explored the effect of combining polyvinylpyrrolidone-co-vinylalcohol (PVP-VA) and Eudragit E (a poly amine). Initially they examined the use of Eudragit E alone, and found that the dispersion displayed two glass transitions above approximately 10-15% drug loading (Six *et al.*, 2003). Following this they produced dispersions which contained both PVP-VA and

Eudragit E, which showed two distinct regions; a PVP-VA-itraconazole region and a Eudragit E-itraconazole region. These dispersions were shown to improve physical stability over binary dispersions and a higher dissolution rate. When high levels of Eudragit E were used a higher apparent solubility was observed, and this did not fall over time (Six *et al.*, 2004). Hydrophobic interactions have been used to entrap paclitaxel in a polymer blend matrix in order to increase drug release (Mu *et al.*, 2005).

1.11 AIMS OF THE THESIS

The overall aims of this thesis are:

- To enhance the understanding of how physical properties of individual polymers affect the dissolution of drugs from dispersions containing combinations of polymers.
- To examine how blends of polymers may be used to increase the amorphous stability of unstable amorphous drugs.
- To investigate the use of near infrared spectroscopy as a method for investigating the amorphous stability of drugs in solid dispersions.

Chapter 2

Materials and Methods

2.1 MATERIALS

Material	Source	Specific Information
Clotrimazole	ICN Biomedicals Inc, Aurora, USA	Product code 198944 Lot 198944
Flavanone	Acros Organics, Geel, Belgium	Product code 146620250 Lot A014658101
Griseofulvin	Sigma Aldrich, St Louis, USA	Product code 856444 Lot 50209015
Indomethacin	Sigma Aldrich, St Louis, USA	Product code 17378 Lot 122K0718
Poly(acrylic acid) M_w 2000	Sigma Aldrich, St Louis, USA	Product code 323667 Lot 20005CA
Poly(acrylic acid) Mw 250 000	Sigma Aldrich, St Louis, USA	Product code 352001 Lot 25003PB
Poly(2-hydroxy-propylmethacrylate)	Sigma Aldrich, St Louis, USA	Product code 529400 Lot TI02008CI
Polyvinylpyrrolidone	Sigma Aldrich, St Louis, USA	Product code 856568 Lots 03042013 and 80717003
Sucrose	Sigma Aldrich, St Louis, USA	Product code S7903 Lot 51K0026
Indium (99.999% purity)	PerkinElmer Instruments, Beaconsfield, UK	Product code 03190033 Lot I439099
Lead (99.999% purity)	PerkinElmer Instruments, Beaconsfield, UK	Product code 03190035 Lot L210603
Zinc (99.999% purity)	PerkinElmer Instruments, Beaconsfield, UK	Product code 03190036 Lot Z250203

Table 2.1 Sources and specific information for materials used in the studies presented in Chapters 3-6.

All other reagents were of analytical grade.

2.2 PRE-SPRAY DRYING SOLUBILITY STUDIES

2.2.1 INTRODUCTION

This work largely focuses on the exploration of dispersions consisting of a poorly water soluble drug with polyvinylpyrrolidone (PVP) and a second excipient both of which would be considerably more water soluble. These dispersions were to be prepared by a co-evaporation method; spray drying. The first aim was therefore to prepare homogenous solutions of drug and excipients. One of the issues that arises from the co-evaporation method has traditionally been that large amounts of solvent have had to be used. For example Chiou and Riegelman (1969b) used 500 ml of ethanol to prepare 5 g of a griseofulvin (10%) in polyethylene glycol (PEG) 6000 dispersion. In order to find solvent systems amenable to the production of homogenous solutions of drug with excipient(s) for spray drying, a number of solubility studies were performed using, in sub-millilitre quantities, a variety of solvents at room temperature.

2.2.2 METHODOLOGY

The saturation solubility of each of the drugs was investigated by the dissolution of an excess of solid (approximately 100 mg) into each of the solvents (water, ethanol and acetone; 1 ml) in a 2 ml glass vial. Where the solid was observed to have dissolved, then ultraviolet (UV) spectroscopy was used to ascertain the concentration of drug in solution. The supernatant solution was filtered using a 0.45 μm polypropylene filter into a fresh 2 ml glass vial. A quantity of the solution (200 μl) was diluted with the same solvent (water ethanol or acetone; to 2 ml) and a scan of the UV spectrum covering 220-400 nm was used to locate the wavelength of λ_{max}. This was performed on a Cary 3E UV-Vis spectrophotometer connected to a PC running Cary WinUV v3.0 (both Varian, Inc., Palo Alto, USA). The average absorption of the sample at the λ_{max} (based on four samples) was calculated. A calibration curve for the drug was then prepared. Trial and error was used to find the approximate amount of drug that equated to the observed absorption. The calibration curve was then produced using drug concentrations covering 0-140% of nominal content. The saturation solubility of the drug was then calculated from the "least squares" linear regression line of the calibration curve calculated using the "LINEST" function in Microsoft® Office Excel 2003.

A stock solution of PVP was produced by using agitation to dissolve PVP (1 g) in distilled water (to 10 ml) in a 14 ml glass vial. A further stock solution was prepared by

dissolving PVP (1 g) in ethanol (to 10 ml). Further stock solutions were then produced by dissolving 1 g of each of the following excipients in water, ethanol and acetone (to 10 ml); sucrose, poly(acrylic acid) (PAA), poly(2-hydroxypropylmethacrylate) (PHPMA), Eudragit L (methacrylic acid-methyl methacrylate copolymer [1:1]) and Eudragit S (methacrylic acid-methyl methacrylate copolymer [1:2]). Studies were also performed to find out the concentration of a saturated solution of each of the drugs (griseofulvin, clotrimazole, indomethacin and the small molecule, flavanone) in acetone, ethanol and water.

Each drug solution was in turn combined with each of the polymer solutions. Each of the PVP solutions was in turn combined with each of the polymer solutions that did not contain PVP. Binary polymer solutions were in turn combined with each of the drug solutions. The drug and PVP binary solutions were in turn combined with each of the remaining polymers solutions. The amount of each solution used was heavily dependent on the nature of the dispersion that was being produced. For example, in order to test whether an indomethacin, PVP and PAA tertiary solid dispersion could be prepared in "Order One" (Section 3.2.5), PVP in ethanol (1 ml, 10% w/v) was added to a stirred solution of PAA in water (100 µl, 10% w/v) in a 2 ml glass vial. The solution was allowed to mix for 2 h. A solution of indomethacin in ethanol (100 µl, 10% w/v) was then added. This mixture was stirred for a further 2 h before being checked for precipitates. The detection of precipitates was performed by visual means against both dark and light backgrounds. The expectation was that by combining solutions, any precipitation would be likely to be rapid and obvious. Sections 3.2.1, 3.2.2, 3.2.3, 4.2.1, 4.2.2, 5.2.1 and 5.2.2 contain further details of the solubility experiments that were performed.

2.3 SPRAY DRYING

2.3.1 INTRODUCTION AND THEORY

Despite being initially developed around sixty years ago, spray drying as a pharmaceutical manufacturing technology is still in its infancy. The temperatures used in spray drying are considerably higher than freeze drying yet spray drying is still considered to be a low temperature technique which can be applied to temperature sensitive materials. Spray drying has two main advantages over freeze drying and traditional oven drying; it is unique in that the drying process produces discreet particles from a feed solution, emulsion or suspension (or a combination). It is also a rapid

process. The spray drying process has been defined as the transformation of feed material from a fluid state into a dried particle by spraying the feed material into a drying chamber (Farid, 2003). The conversion of the feed liquid into a powder is a single process. Dependant on the spray dryer design and feed system, this powder may have the virtue of being well defined in terms of particle size and morphology.

Atomisation of the feed into a fine spray of droplets is considered to be the first stage of spray drying. At this point, there are a number of parameters that can affect the product characteristics. A homogenous spray must be produced to ensure that optimum evaporation occurs and that the product has the desired particle size, shape and residual moisture. A number of different nozzle systems can be used to create the atomised solution. The nozzle system described in the instrumentation section below is that which was used throughout this body of work. The atomised solution then comes into contact with the drying gas. The drying gas is typically air though it may be another gas if the system is a closed one, that is to say a system in which the gas is drawn from a nitrogen concentrator or similar.

Post atomisation, the actual drying then takes place as the liquid component(s) are removed. Over time, five main models have been proposed for drying. Before discussing these models an explanation of the wet bulb temperature (T_{wb}) is required as this will be referred to later. When a solvent evaporates, it absorbs heat energy from the surrounding air in order to change phase (this is related to the enthalpy of vaporisation), thus reducing air temperature slightly. The T_{wb} varies with relative humidity; if the relative humidity is quite low whilst the temperature is high, moisture will evaporate quickly thus its cooling effect will be more significant than if the relative humidity was already high, which would reduce the rate of evaporation. A practical example of this effect would be to measure room temperature with a thermometer (measuring dry bulb temperature, T_{db}) and then re-measure it having wrapped a damp piece of cloth around the thermometer. The temperature the thermometer measures will be lower when wet then when dry.

Initially it was assumed that the droplet was in essence to be at a uniform temperature, with the feed solvent diffusing through the particle and evaporating at the surface. A diffusion equation based on effective diffusivity (rather than molecular diffusivity) was used to predict drying (Wijlhuizen *et al.*, 1979). A later approach considered the

possibility of the formation of a crust that increased in depth as the damp core receded (Nesic, 1989). Again the assumption was that temperature was constant across the particle. This was refined when Cheong and co-workers (1986) considered a receding core which differed in temperature to the crust, yet the core temperature was not linked to the T_{wb}. Chen and Xie (1997) took a slightly different approach. They ignored temperature and solvent distribution and their (aqueous) model focused on the loss of water from a droplet which steadily caused a reduction in the rate of drying as water vapour pressure dropped. It proved to be an accurate model for drying but did not aid prediction of droplet temperature.

A more recent model, a diagram of which is shown in Figure 2.1 that tries to overcome these limitations was proposed by Farid (2003). His system fits rather neatly into four separate stages outlined below:

1. The particle is heated until it reaches the T_{wb}. There is no change in the size or the shape.
2. The particle starts to shrink but it still remains at the T_{wb}. This is confirmed by a number of studies (Wijlhuizen *et al.*, 1979; Nesic, 1989). This continues until the crust is rigid enough to hold the shape of the particle.
3. At a critical depth the rigid crust prevents shrinkage from continuing, yet evaporation continues with two defined regions, an inner wet core, which is ever-decreasing in size and an outer dry crust ever increasing in size. The particle stays at the wet bulb temperature.
4. After drying is completed either because there is no more solvent or the solvent is locked in the core there is a slight heating of the droplet

The final step of the spray drying process involves the separation of the dried product from the drying medium. This usually occurs in a cyclone, which uses centrifugal forces to separate particles by size. Intermediately sized particles will drop straight out of the cyclone into a collecting jar, whilst larger particles will be thrown around the cyclone until they are reduced to an intermediate size and drop out of the cyclone into the collecting jar. The very smallest particles (often called the fine particle fraction or fines for short) will be least affected by the cyclone and pass straight through it to be collected in a filter. On some systems this fraction is recoverable.

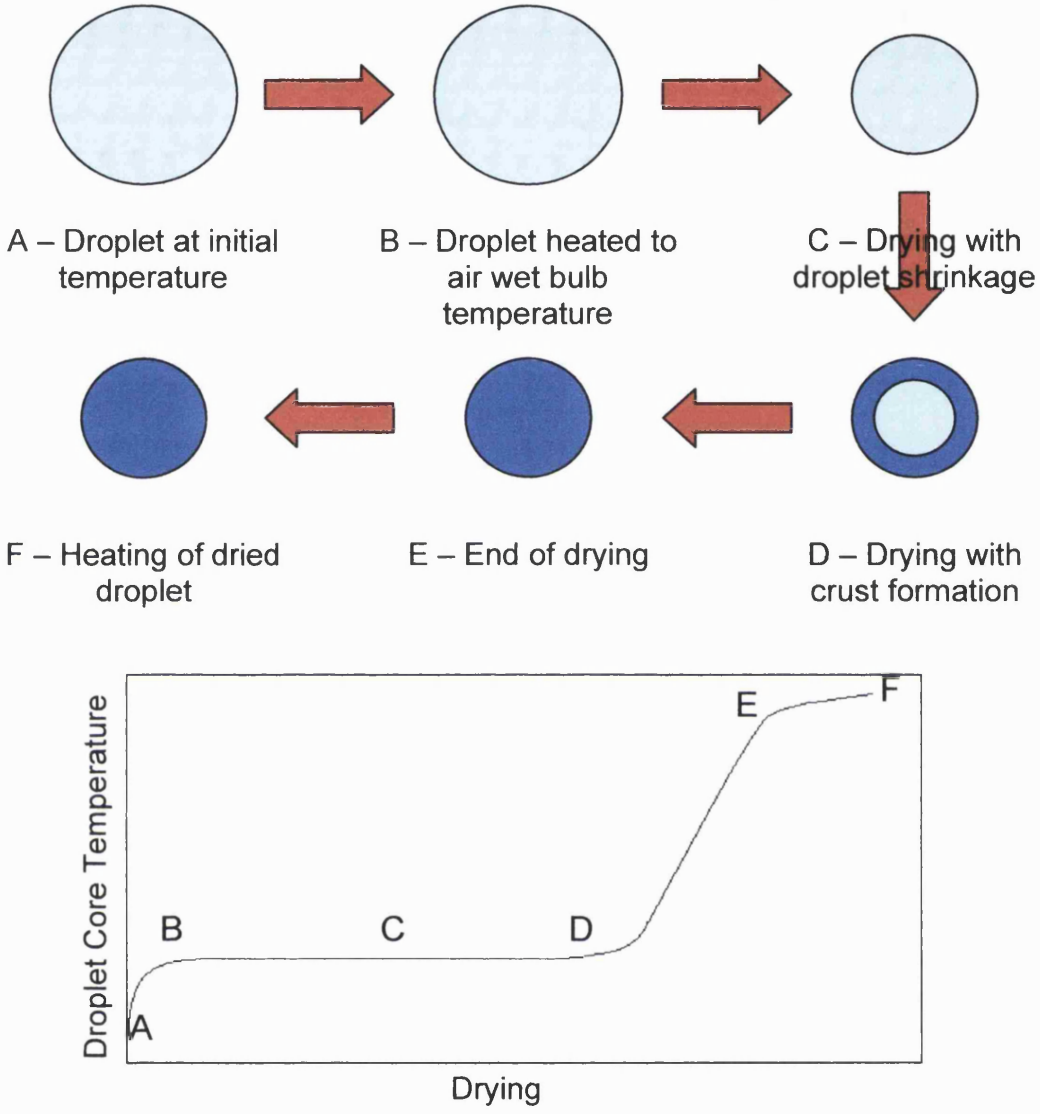

Figure 2.1 The drying of a single droplet in the spray dryer and its core temperature. Adapted from Farid (2003).

2.3.2 INSTRUMENTATION

The Niro SD Micro system (Niro Group, GEA Powder Technology Division, Søborg, Denmark) was used to spray dry feed solutions. A diagram and photograph of system are shown in Figure 2.2. The Niro has two main advantages over many other pilot-plant spray dryers. It is connected to a nitrogen generator to permit the use of flammable organic feed solutions, and the fluid dynamics mimic those of an industrial scale plant, therefore scale up should not be an issue. The system operates with a two fluid pneumatic nozzle that induces atomisation by shattering a liquid film of spray with a high velocity gas. The gas creates frictional forces over the liquid surface which causes

the liquid to disintegrate into spray droplets. This process is named bi-phasing and can be sub-divided into two steps. Initially the liquid is divided into filaments and large droplets. Following this the atomisation process is concluded by shearing the filament or large droplets into small droplets. The Niro uses a so called co-current system in which the spray and the air come from the same side. In a co-current system the particles usually reach a temperature of approximately 10°-15 °C below the spray dryer inlet temperature and are exposed to this temperature for a maximum of 30 s. Therefore very heat sensitive systems can be dried as the evaporation is very rapid.

2.3.3 METHODOLOGY

A feed solution containing dissolved solid (3-10% w/v) was prepared in a conical flask (usually 250 ml, occasionally 500 ml), using a magnetic stirrer, with dissolution being confirmed by visual inspection only. The rates of nitrogen entering the drying chamber of the Niro were set to 20 kg/h for the high velocity shattering gas and 2 kg/h for the drying gas. The inlet temperature was set to around 65 °C though this varied depending on the solvent system used. The furnace was then turned on. When the inlet temperature reached 10 °C over the set temperature, the peristaltic pump controlling the spray of feed solution was turned on. It was typically set to a rate of 20% of maximum which equates to approximately 3 ml/min. The target outlet temperature was set to around 20 °C below the inlet temperature. A mixture of solvents comparable to that in the feed solution was sprayed until the atomisation of liquid was consistent. Consistent atomisation was required to produce well defined particles. Once this was reached the hose to the pump was transferred to the feed solution. The main product was collected in a previously weighed jar under the cyclone whilst the fine part was not collected. Care had to be exercised during each run to ensure that the feed nozzle did not become blocked with solid. Once a run had been completed, the hose to the peristaltic pump was transferred back to the clean solvents and the jar removed from the system to prevent wetting of the particles. After 24 h drying in a vacuum oven at room temperature the jar was weighed again to provide an accurate assessment of yield.

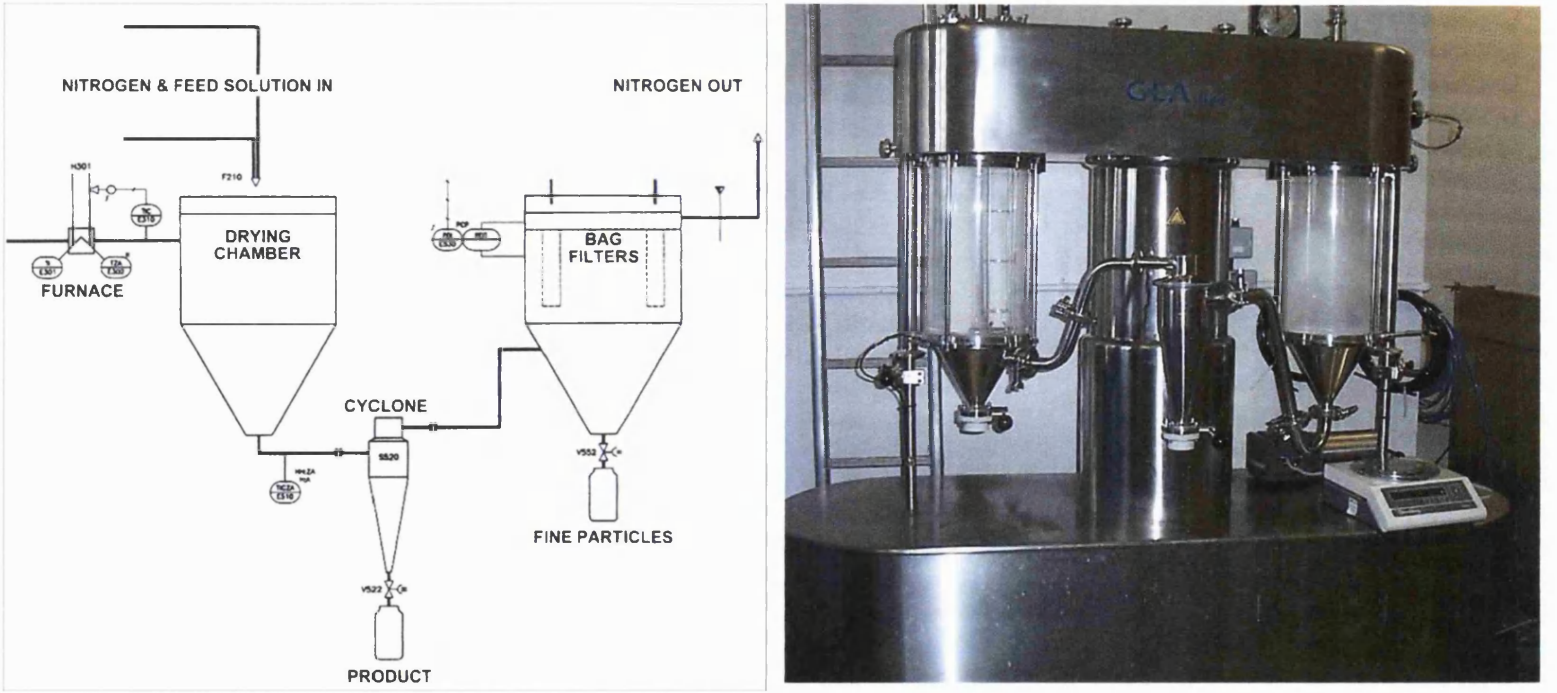

Figure 2.2 A schematic and a photograph of the Niro SD micro spray dryer. The schematic is a mirror image of the photograph, as conventionally schematics flow from left to right.

2.4 THERMOGRAVIMETRIC ANALYSIS

2.4.1 INTRODUCTION

Thermogravimetric analysis (TGA) is a technique used to measure changes in the mass of a sample as a function of temperature or time. It is performed by loading a sample (of known weight) on to a mass pan. The sample then undergoes a heating step at a constant rate and the resultant mass change is plotted against temperature or time. Temperature is monitored via a thermocouple that hangs beside the sample pan. It controls the heating element of the TGA which consists of a cylinder which surrounds the sample and a water-based heat exchanger.

Mass loss occurring during the TGA run can generally be attributed to the loss of solvents (typically water) and other volatile chemicals, or the sample undergoing chemical reactions such as degradation. In order to minimise degradation, the experiment is usually performed in an oxygen free environment, typically with flowing dry nitrogen. This also helps reduce moisture take-up by hygroscopic samples.

2.4.2 INSTRUMENTATION

Thermogravimetric analysis was performed using a TA Instruments Hi-Res TGA 2950 ThermoGravimetric Analyser (Figure 2.3) and analysed using Thermal Analyst 2000 v1.0 (both TA Instruments, Inc. Leatherhead, Surrey UK).

2.4.3 METHODOLOGY

A PerkinElmer non-hermetic pan base was loaded onto the mass stirrup of the TGA and tared. The pan base was then loaded with 10 to 20 mg of sample, evenly spread, but not compressed across the base. This was then loaded onto the TGA and the TGA furnace was raised. A typical heating run would consist of a hold step at 30 °C lasting for 1 min to allow the sample to equilibrate, followed by a linear heating step to a temperature of at least 150 °C at a rate 10 °C/min. Following this, the sample was automatically removed from the furnace and the furnace was cooled by compressed air. Unless otherwise stated, the experiment was repeated in triplicate and the average mass loss calculated.

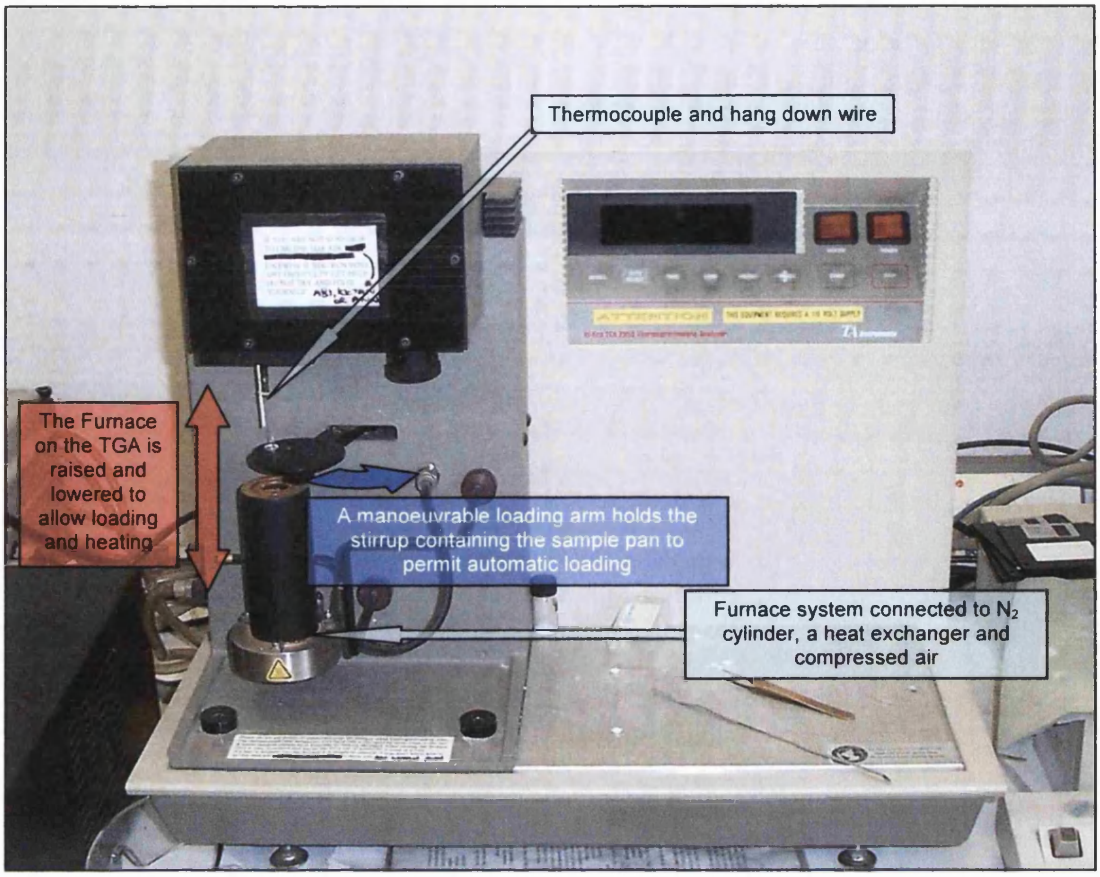

Figure 2.3 An annotated photograph of the TA Instruments Hi-Res TGA 2950 ThermoGravimetric Analyser.

2.5 Differential Scanning Calorimetry

2.5.1 Introduction

Power compensation differential scanning calorimetry (DSC) is performed by applying the same thermal conditions, typically a heating scan at a consistent rate, to a sample pan and a reference pan, each of which is heated by a separate furnace. The difference in the energy required by each furnace is then recorded against temperature or less commonly time. As the sample undergoes a transition, energy will be absorbed or evolved by the sample. A feed-back loop causes the energy input by the sample furnace to change and this is measured to provide a direct calorimetric measurement of the transition energy. This technique is called the power compensated "null-balance" DSC principle. The technique can be used to detect and quantify many transitions and reactions including sublimation, fusion and vaporisation which will be displayed as endothermic events, crystallisation (exothermic) and glass transitions, which result in a baseline shift as the specific heat capacity of the sample alters.

2.5.2 INSTRUMENTATION

A DSC 7 Differential Scanning Calorimeter (PerkinElmer Instruments, Beaconsfield, Bucks, UK) was used. It is based on a power compensated "null balance" DSC principle, as outlined above. Platinum resistance heaters and temperature sensors are used to make the temperature and energy measurements. The DSC 7 was equipped with a refrigeration unit to permit sub-ambient operation to around 0 °C and rapid cooling to provide quick sample turnaround and a built in glove box to prevent build up of ice on the DSC. The DSC 7 was controlled by an IBM-compatible PC running the Pyris Thermal Analysis Software (version 3.81) for Windows via a thermal analysis controller TAC 7/DX.

2.5.3 METHODOLOGY

Each sample (1.5-8 mg) was weighed to two decimal places into a non-hermetically sealed DSC pan which was then crimped and placed on to the sample furnace. An empty pan was also crimped and this was placed on to the reference furnace. A typical DSC scan of the sample was then made. This consisted of an initial isothermal hold for at least one minute followed by a temperature scan at 10 °C/min over a range that covered all the expected transitions. On completion of a temperature scan, analysis was performed using the Pyris Thermal Analysis Software (version 3.81) for Windows.

2.5.4 CALIBRATION

Calibration was performed at a minimum of once a fortnight. Calibration also took place whenever the scan rate was altered, whenever the equipment was switched off or whenever there was any doubt about the validity of the current calibration.

Very high purity (99.999%) metal standards were used to calibrate the temperature and ΔH values. Indium and either zinc or lead were used to calibrate temperature, whilst indium alone was used to calibrate the change in enthalpy (ΔH). Onset temperatures and transition energies are given in Table 2.2. To confirm the validity of the calibration an indium run was performed after the calibration. Fresh zinc or lead samples were used each time. Fresh zinc was used because it alloys with aluminium on melting, whilst lead is very readily oxidised. Indium samples were reused up to five times.

Standard	Onset T_m °C	Transition energy in J/g
Indium	156.60	28.45
Lead	327.47	23.01
Zinc	419.47	108.37

Table 2.2 The theoretical onset temperature of melt and transition energy for each of the standards used to calibrate the differential scanning calorimeters.

2.6 HYPER DSC

2.6.1 INTRODUCTION

High-speed DSC and hyper DSC (also High Performance DSC and HPer-DSC) are interchangeable blanket terms covering the use of heating rates typically in excess of 100 °C/min and as much as 500 °C/min, where traditionally DSC has covered a range of about 1 °C/min to 20 °C/min. This provides a number of advantages, the most pertinent of which is that hyper DSC can be used to prevent recrystallisation during a heating step (Pijpers *et al.*, 2002). DSC results are most commonly displayed as milliwatts (mW; in effect millijoules per second) against temperature and consequently, by decreasing the time over which energy can be absorbed or evolved one can increase the size of a peak or baseline shift. This is particularly useful for amorphous systems in which the ΔCp is quite small, or where the amorphous content is quite low (Saunders *et al.*, 2004). Unfortunately samples which undergo transitions that are heavily time dependent are less amenable to hyper DSC experiments. This is an issue for samples that contain small amounts of solvent as the loss of solvent can mask glass transitions and small melting steps.

2.6.2 INSTRUMENTATION

A Pyris 1 Differential Scanning Calorimeter (PerkinElmer Instruments, Beaconsfield, Bucks, UK) was used for Hyper DSC. It differs from the DSC 7 by virtue of having much smaller furnaces which reduce thermal lag and provide more rapid heating and cooling. This permits modulated scans and rapid scan rates. The Pyris 1 is equipped with an Intracooler 2 refrigeration unit permitting sub-ambient operation down to around -50 °C. The Pyris 1 was controlled by an IBM-compatible PC running the Pyris Thermal Analysis Software (version 3.81) for Windows via a thermal analysis controller TAC 7/DX.

2.6.3 METHODOLOGY

In essence the experimental technique for Hyper DSC was the same as that for slower rate experiments (Section 2.5.3). The amount of sample used was generally smaller (1-2.5 mg) in order to reduce the effect of thermal lag in the sample. The sample mass was accurately weighed into a non-hermetically sealed DSC pan. This was crimped and placed on the sample furnace. An empty pan was crimped and placed on the reference furnace. The system was brought to equilibrium with an isothermal hold of a minute. This was followed by a heating step at 200 °C/min covering all the expected transitions (typically from 20 °C to 200-220 °C). Further cooling, holding and heating runs were often performed on raw materials in order to better characterise the glass transition temperature (T_g). Calibration was performed as per Section 2.5.4. Data analysis was performed using the Pyris Thermal Analysis Software (version 3.81) for Windows.

2.7 X-RAY POWDER DIFFRACTION

2.7.1 INTRODUCTION

X-ray powder diffraction (XRPD) is a technique most often used to assess the crystalline or amorphous nature of bulk powder. In essence XRPD is performed by firing a stream of X-rays (α_1-particles) at a sample and measuring the angle at which they bounce off (are diffracted). Crystalline material creates a consistent set of diffraction angles of X-rays whilst amorphous material causes random diffraction of X-rays which produces a "halo" effect. Within reason it is possible to gauge the degree of disorder of a system quantitatively by measuring characteristic peak intensities or peak areas (most commonly integrated using a lorenzian distribution), and comparing these with samples with known disorder (Black and Lovering, 1977). However, in systems which are 90-100% crystalline it often becomes very difficult to detect the differences between these systems. In highly crystalline systems the sensitivity decreases due to the technique measuring the diffraction of X-rays in a bulk sample (Saleki-Gerhardt *et al.*, 1994). Difficulties are encountered in highly amorphous systems due to the non-linear nature of the base line and the level of noise encountered, which can limit detection and quantification of peaks to a minimum of 5% (Clas *et al.*, 1995). Hurst *et al.* (1997) identified a total of 33 parameters that could affect the accuracy of quantitative XRPD, which were assigned into one of three categories. These are presented in the following tables adapted from their paper (Table 2.3a, 2.3b and 2.3c).

Instrument or systemic parameters	**Notes**
X-ray source intensity	Increasing kV and mA can increase both peak and background intensity and improve counting statistics
Scan rate	Reducing scan rate may improve the difference between peaks and background noise as noise is a random occurrence. Chopper increment relates to the total 2Θ-interval over which counts are averaged
Chopper increment (or Step size)	
Divergence slit width	At low °2Θ with too wide a slit the sample is only partially hit by part of the beam. Yet at higher angles less of the sample surface is irradiated with too narrow a slit
Receiving slit width	If the sample is granular a wider slit needs to be used to collect all the relevant peak detail, however this can decrease resolution
Detector dead time	Results from measured count rate not being directly proportional to photon rate, this can cause an increase in intensity for weaker peaks
Lorentz-Polarisation factor	X-ray beams are polarised to a varying extent depending on the angle of scatter. The lorentz-polarisation factor can compensate for this but not at extreme angles
Counting statistics	If the height of a peak (number of counts) is quite small slight variations in number of counts due to background noise can have a large effect
Total surface irradiated	The larger the surface area over which analysis takes place, the better the quantitative accuracy
Sample displacement error	The measured peak position and to an extent, the diffraction intensity can be affected by the sample being positioned at a slight angle, or having an uneven surface. This effect is especially pronounced at low °2Θ
$K_{\alpha 2}$ stripping	At low °2Θ α_1 and α_2 peaks overlap which whilst at high °2Θ this does not occur. One can strip α_2 peaks via data manipulation but this will have an effect on peak intensity.
Data smoothing	Data can be smoothed but this will affect peak intensity
Background manipulation	All can effect accuracy
Method of Peak area measurement	
Software manipulation	

Table 2.3a Instrumental and systematic parameters affecting the accuracy of XRPD measurements.

Inherent properties of the phase	**Notes**
Structure factor	This is determined by the types of atoms contained in an ordered structure and the coordinates of each in the unit cell
Multiplicity	A crystal has a minimum of two faces however this can be much higher with some faces having the same reflections
Crystallinity or amorphous nature	Heavier atoms with no (or only short range order) may be masked by lighter atoms that have long-range order. Areas of short range order can produce low, broad peaks which may affect the baseline. When peaks which are to be analysed show this kind of effect there is a risk of large analytical error
Absorption factor (μ)	If μ is quite small the X-rays will penetrate further into the sample body effectively causing sample displacement error. Should the sample be quite thin, the X-ray scan taken will not be accurate
Primary extinction	Primary extinction occurs when an already diffracted beam is reflected, refracted or diffracted back at incident beam and destructively interferes due to it being out of phase. Secondary extinction occurs when one crystal lies below another and so is hit by a less intense beam
Secondary extinction	
Temperature	As temperature increases there is an increase in molecular mobility causing expansion of the unit cell, decreasing diffraction intensities and increasing background scatter

Table 2.3b Inherent properties of the compound or material that affect the accuracy of XRPD measurements.

Sample preparation effects	Notes
Primary and secondary extinction	See Table 2.3b
Size distribution of coherent diffraction domains (CDD)	Coherent diffraction domains are considered to be at most a complete crystal but may only make up part of a crystal or particle. The size of a CDD has an effect on intensity with a maximum intensity being achieved at about 100 to 5000 repeat units (0.1-5 μm). Larger CDDs tend to have increasing primary and secondary extinction. Whilst smaller CDDs tend to lack a strong structural factor, or cause peak broadening which is indistinguishable from background noise at less than 0.005 μm
Preferred orientation	This occurs when sample preparation causes a particular orientation of crystallites. It is probably the largest single source of error and will be discussed below
Sample homogeneity	In dry mixing it is very easy to produce uneven mixes. Whilst variation in particle size can have an effect akin to the CDD effect outlined above
Average mass attenuation coefficient ($\tilde{\mu}$)	Samples with a low $\tilde{\mu}$ will allow more X-rays to pass through them (like light through glass), this increases the risk of displacement errors, primary and secondary extinction and X-ray interaction with the sample holder
Micro-absorption	This occurs when samples contain elements with different mass absorption coefficients are analysed together
Size of exposed sample surface	As 2Θ changes the area of sample irradiated changes, if this begins to spread to areas outside the sample holder, errors can creep in
Thickness of powder	The powder bed needs to be think enough to prevent any X-rays from passing through it with the minimum thickness being related to $\tilde{\mu}$ and °2Θ
Sample surface characteristics	Any curving, roughness or porosity can cause peak displacement.
Powder packing and transparency	Packing is required to reduce porosity and roughness however it can induce preferred orientation and may change the level of amorphism
Method for producing sample mounts	Smear mounting can cause an increase in preferred orientation which is less of an issue with back-mounting
Choice of internal standards	Standards need to be selected for reproducibility, and should have peaks near those in the analyte

Table 2.3c Parameters related to the preparation of samples that affect XRPD results.

Studies into the effect of rotation and particle size reduction on the preferred orientation effect on samples of mannitol polymorphs showed that reducing the particle size can improve XRPD accuracy by reducing the effect of preferred orientation and this was found to be more effective than merely rotating a sample. In combination slight benefits were seen in day-to-day reproducibility, instrument reproducibility and intra-day reproducibility, sample positioning and sample packing, with the main benefit being associated with sample mixing (Campbell-Roberts *et al.*, 2002).

2.7.2 INSTRUMENTATION

A Philips PW37010 X-ray Powder Diffractometer (Philips, Cambridge, UK) was used to the analyse samples. An annotated photograph of this diffractometer is shown in Figure 2.4. A copper anode contained within an X-ray tube is bombarded with electrons to produce the X-ray α_1-particles which then exit the tube through a beryllium window. The X-rays then pass through a series of 1 mm divergence slits which focus the X-rays onto the sample, which is held on a non-spinning stage. Here they are diffracted by the sample to varying degrees. A detector arm which moves in an arc collects X-rays which diffract into its path. Before hitting the detector the X-rays pass through a number of slits to filter out X-rays travelling along a different angle to that of the detector. The detector then delivers an output of intensity versus °2Θ.

2.7.3 METHODOLOGY

The sample (180 mg to 240 mg) was loaded into a circular well in the XRPD sample holder which had a diameter of approximately 260 mm and a depth of approximately 1.5 mm. This was then compressed to produce a flat surface before being loaded into the XRPD. The sample was then scanned using an X-ray lamp tension of 45 kV and 30 mA. Typically, a continuous scan was performed between 15.5 °2Θ and 29.5 °2Θ, with a step covering 0.02 °2Θ and a time per step of 2 s. The angles between which the scan took place varied according to the sample used however the other parameters remained constant. Computer analysis was performed using PANalytical X'Pert HighScore v2.0a. Background analysis was performed using a rolling average system (Sonneveld and Visser, 1975). Following this peaks were located that had a minimum significance of 0.7, a minimum tip width of 0.1 °2Θ and a maximum tip width of 1 °2Θ. Peak height was then integrated assuming a Lorenzian distribution. Unless otherwise stated samples

were assayed a minimum of three times and were randomly assigned either repacking and/or rotation between repeats.

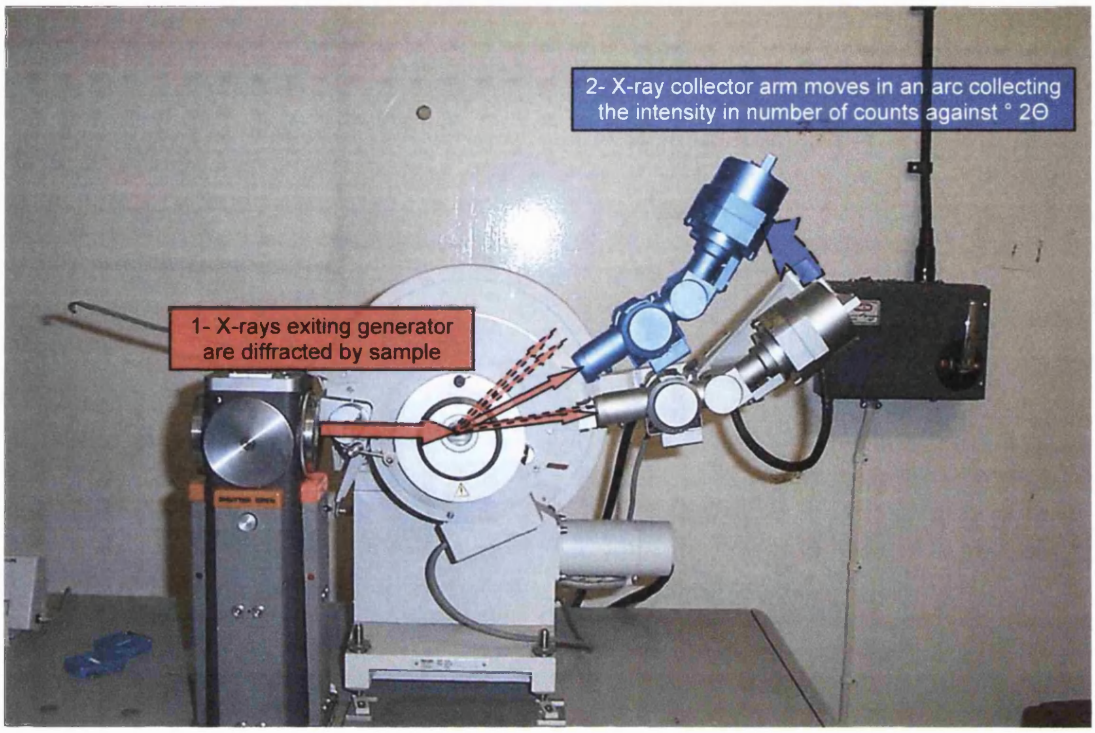

Figure 2.4 An annotated photograph of the Philips PW37010 X-ray Powder Diffractometer demonstrating the diffraction of X-ray particles by a sample and their subsequent detection.

2.8 Infrared Spectroscopy

2.8.1 Introduction

The region of the infrared (IR) spectrum which is of greatest interest to organic chemists is the wavelength range 2.5 to 15 μm. In practice, units proportional to frequency, (wave number in units of cm^{-1}), rather than wavelength, are commonly used and the region 2.5 μm to 15 μm corresponds to approximately 4000 cm^{-1} to 600 cm^{-1}. This is also known as the mid-infrared spectrum. In any given bond between two molecules there are a number of vibrational motions that can occur. In order for any particular vibrational motion to occur the molecules must absorb infrared radiation of a specific wavelength. In theory, one can therefore fire an IR beam at a sample and measure which wavelengths are absorbed in order to identify which functional groups are present. Attenuated total reflection is a technique which does not require one to pass the IR beam through a sample. Instead, the IR beam is reflected through an optically dense

(having a high refractive index) IR transmitting prism, such as a germanium crystal. The resulting internal reflection creates an effect called the evanescent wave that extends beyond the surface of the crystal into the sample that is in contact with the crystal. In regions of the infrared spectrum where the sample absorbs energy, the evanescent wave will interact with the sample and a spectrum can be obtained.

2.8.2 INSTRUMENTATION

Samples were collected with a Nicolet Avatar 360 ESP (Thermo Electron Corporation, Waltham, Massachusetts, USA) fitted with a germanium crystal stage which enables one to take samples using the attenuated total reflectance infrared technique. In the attenuated total reflectance infrared technique, infrared radiation from the spectrometer enters the germanium crystal and is reflects through the crystal and penetrating into the sample a finite amount with each reflection along the top surface via a so-called "evanescent" wave (Omnic, 1998). At the output end of the crystal, the beam is directed out of the crystal and back into the normal beam path of the spectrometer. Analysis was performed using Nicolet Omnic v5.0 (Thermo Electron Corporation, Waltham, Massachusetts, USA). The Avatar 360 is a Fourier-transforming infrared spectroscope (FTIR).

2.8.3 METHODOLOGY

The sample (1-2 mg) was placed in the centre of the crystal. The sample was then scanned 32 times to produce a single spectra. An average spectra was produced using three such sample spectra.

2.9 NEAR INFRARED SPECTROSCOPY

2.9.1 INTRODUCTION

The near infrared (NIR) spectrum covers an area of around 1100 nm (sometimes extended to 750 nm) to 2500 nm, and is just above the visible spectrum. Unlike the mid infrared region which is produced by the absorption of specific bonds, it is overtones and combinations of bonds that contribute to NIR spectra (Bugay, 2001). The use of the near infrared region to study pharmaceutical products is less well developed than other usages but NIR does have the virtue of requiring little sample preparation and is rapid, non-destructive and non-invasive. Recently developed techniques involve the determination of the degree of crystallinity within physical mixes of crystalline and amorphous substances (Seyer *et al.*, 2000) and the detection of polymorphism and

changes in physical form (Aldridge *et al.*, 1996; Buckton *et al.*, 1998; Fevotte *et al.*, 2004). Traditionally the NIR spectrum has been considered to be more difficult to work with than the mid-infrared, due to the innate variability of peaks which are due to overtones and combinations. With the aid of computer analysis, results are now more amenable to analysis, especially through mathematic manipulation such as chemometrics, principal component analysis (PCA) or simple 2nd-derivatives of the spectra. The entire NIR spectrum has been well characterised, thus spectral information can be extremely useful.

2.9.2 INSTRUMENTATION

Samples were collected with a Foss NIRSystems rapid content sampler (Foss NIRSystems, Maryland, USA) and a Foss NIRSystems model 6500 spectrophotomer (Foss NIRSystems, Maryland, USA) connected to a computer running Vision® for Windows version 2.51 (Foss NIRSystems, Maryland, USA).

2.9.3 METHODOLOGY

A sample mass of around 1 to 1.5 g was placed in a 4 ml Waters vial. The vial was then sealed with Parafilm. The Parafilm was pierced and the sample placed under continuous vacuum for a minimum of 24 h. The vial containing the sample was shaken and tapped before being placed on the sampling unit and an average of 32 spectra taken. The shaking and sampling was then repeated twice to produce an average spectrum.

For direct comparison of one spectrum to another the Vision® software was used to produce standard normal variance (SNV) 2nd-derivative spectra. SNV is a technique whereby the average value in the spectrum is calculated and subtracted from all the spectral values in turn. It is used to remove a baseline shift. These SNV 2nd-derivative spectra were imported into a chemometric software package; The Unscrambler® version 7.6. This software uses a number of algorithms to perform multivariate data analysis. In essence this is the use of formulas and algorithms to calculate which areas of a data set give rise to the greatest amount of variance in that data set. This is discussed further in Sections 6.2.4 and 6.2.5.

In general, routine calibration was not performed beyond the use of a ceramic reference disc which was used to produce a background against which samples could be run. A new reference scan was taken on the same day as any sample scans were taken. Owing

to the inherent variability of NIR, all samples taken via this method were performed using the same lamp. As the lamp has a limited life, a performance test was performed each time the system was used. This is an internal validation test that checks the instrument parameters such as noise, bandwidth, and wavelength accuracy.

2.10 Dynamic Vapour Sorption and Near Infrared Spectroscopy

2.10.1 Introduction

Dynamic Vapour Sorption (DVS) and Near Infrared Spectroscopy (NIRS) is a combined technique which provides physical and chemical data on the effects of controlled relative humidity conditions on a sample. DVS is a gravimetric technique, primarily used to investigate water uptake or loss at tightly controlled relative humidities. The DVS/NIRS technique was initially described by Lane and Buckton (2000) and has since been used to examine crystallisation and polymorphic changes in lactose which permitted the quantification of very low levels of amorphous material (Hogan and Buckton, 2001a), raffinose and salbutamol, both of which showed unusual crystallisation transitions (Hogan and Buckton, 2001b; Columbano *et al.*, 2002) and theophylline, which showed a transition not seen before (Vora *et al.*, 2004).

2.10.2 Instrumentation

All experiments were performed on an adapted DVS-1 system (Surface Measurement Systems, London, United Kingdom) fitted with an optical reflectance NIR probe (Foss NIRSystems, Maryland, USA). The DVS consists of 2 identical flat-bottomed quartz weighing pans suspended from a microbalance (accurate to ± 0.0001 mg) which is housed inside a controlled temperature unit. The pans themselves are housed within separate glass chambers. Software controls and varies the influx of dry nitrogen and saturated wet nitrogen into these chambers, allowing one to accurately control RH between 0 and 98% RH. The changes in RH are programmed into the software either as time controlled steps or as mass change over time limits, which control the movement of one step to another.

2.10.3 Methodology

An NIR probe was positioned approximately 4 mm below the base of the sample pan, and an average of 32 spectra taken at regular intervals using the Vision® software version 2.21. The data set was then displayed as 1/R where R is reflectance. A sample

mass of between 25-50 mg was used and the pans were cleaned with distilled water and absolute alcohol between runs.

2.10.4 CALIBRATION

NIR calibration is discussed in Section 2.9.3. DVS microbalance calibration was performed a 100 mg Class M calibration weight. Relative humidity validation was performed using saturated salt solutions of sodium chloride and lithium chloride.

2.11 *IN VITRO* DISSOLUTION STUDIES

2.11.1 INTRODUCTION

The drug dissolution from spray dried micro-particles was assessed using *in vitro* dissolution tests. Along with dosage form optimisation, dissolution tests can provide early indications of bioavailability, as a drug must be in solution before it can be absorbed. The concentration of drug present in the dissolution media was assessed by ultraviolet (UV) spectrophotometry.

2.11.2 INSTRUMENTATION

The dissolution apparatus used, Pharma Test model PTW S3C (Pharma Test, Hainberg, Germany), conforms to the Apparatus 2 standards laid out in the United States Pharmacopeia and National Formulary 24 (2000). *N.b.* the American form of pharmacopoeia is spelt pharmacopeia. UV analysis was performed using a Cary 3E UV-Vis spectrophotometer connected to a PC running Cary WinUV v3.0 (both Varian, Inc., Palo Alto, USA).

2.11.3 METHODOLOGY

The tests were performed in 900 ml of deoxygenated dissolution media which was maintained at 37.0 ± 0.5 °C with a paddle rotation of 100 ± 2 rpm. The dissolution media used were produced as per the British Pharmacopoeia (2005a). The test was performed on between 10-20 mg of spray dried microparticles that had been accurately weighed into hard gelatine capsules (size 1). A sample (3 ml) of the dissolution media was taken in situ immediately before the start of the dissolution experiment. The capsules containing spray dried microparticles were then dropped into the dissolution media and the timer started. A further 12 samples were collected at predetermined time points using a Pharma Test automated sampler, model PTFC-1 (Pharma Test, Hainberg, Germany). Each sample was 3 ml in size and the volume was not replaced meaning that

across the course of the test 39 ml of dissolution media was lost. This effect was accounted for during the analysis of samples.

Each dissolution test sample was taken from dissolution test apparatus and transferred to one of a matched pair of quartz cuvettes (10-mm path length). The other cuvette was used as a blank and was filled with the same media as was used in the dissolution study. The absorbance at the literature value for the λ_{max} of the drug in question was taken and this was subtracted from the absorbance value of the percentage of drug released was calculated using calibration curves which were consistent with Beer's law and an average of 4 runs was plotted against time. A worked example is presented in Section 3.3.4.

2.12 Scanning Electron Microscopy

2.12.1 Introduction

Scanning electron microscopy (SEM) is a technique that enables the visualisation of surface structures down to sizes as small as 1 μm. It is used to size very small particles and to examine surface characteristics such as roughness. However such observations may be subjective and rely on sample homogeneity. Scanning electron micrographs produced using this technique may appear to be almost three dimensional due to the large depth of field the technique allows. This makes it particularly useful in the analysis of particle morphology and crystal form.

2.12.2 Instrumentation

An Emitech K550 sputter coater (Emitech, Kent, UK) was used to coat the samples with gold to make them electrically conductive. A Philips L20 SEM (Philips, Eindhoven, Netherlands) was then used to obtain scanning electron micrographs.

2.12.3 Methodology

The sample powder was mounted onto an adhesive carbon disc attached to an SEM stub before being coated with gold by sputtering. The sample was then loaded into the SEM to obtain scanning electron micrographs of the sample with the voltage and scale of each micrograph being listed on the individual micrograph.

Chapter 3

The Dissolution of Spray Dried Dispersions Using Indomethacin as a Model Drug

3.1 INTRODUCTION

In studying the effect of the addition of secondary polymers on the properties of solid dispersions/solutions one important property to consider is dissolution. It has been hypothesised that the dissolution of drug from a solid dispersion/solution is, in many cases, dependent on the inherent solubility of the polymers (Craig, 2002). This is partly due to the mechanism by which polymers dissolve, which is currently theorised to consist of the diffusion of solvent into the polymer matrix which results in the polymer changing from being the glassy state to the rubbery state. This facilitates chain disentanglement and (eventually) results in dissolution (Miller-Chou and Koenig, 2003).

A good example of polymer dissolution rate affecting drug dissolution would be poly(ethylene glycol) (PEG). It has been suggested that a change in the polymorphic form of PEG from being linear to once-folded and then to twice-folded could reduce the dissolution rate of indomethacin from PEG dispersions (Ford and Rubinstein, 1979) and this has been demonstrated with glybornuride, which is used to treat diabetes (Vila-Jato *et al.*, 1986). The dissolution rate of a number of drugs including chloramphenicol, paracetamol and indomethacin molecularly dispersed in PEG 6000 was compared and found to be statistically the same (Dubois and Ford, 1985). This suggests that the rate of drug dissolution is dependent on the carrier. The physical stability of solid dispersions of UC-781, an antiviral agent with PEG M_w 6000, Gelucire 44/14 and PVP K30 were analysed by differential scanning calorimetry (DSC) and X-ray powder diffraction (XRPD). Dissolution studies were conducted as a function of storage time. DSC curves of UC-781-PVP dispersions did not show any melting peaks corresponding to UC-781 after storage, indicating no recrystallisation of the drug. The DSC data for PEG 6000 and Gelucire dispersions showed an increase in the enthalpy of fusion of the carriers indicating the reorganisation of the crystalline domains of the polymer. As with DSC, XRPD data did not show any changes in crystallisation and yet dissolution of UC-781 from all solid dispersions decreased as a function of storage time. The research group concluded that a change in the physical state of the carrier and not that of the drug was responsible for the decreased dissolution properties of the solid dispersions (Damian *et al.*, 2002). Tanno *et al.* (2004) showed that the release of nifedepine from hypromellose acetate-succinate into solutions with different pHs could be altered by varying the ratio of succin`oyl and acetyl moieties in the polymer chain, with an increased level of succinoyl moieties increasing dissolution at low pH whilst being detrimental to release at high pH.

It therefore follows that the addition of a second polymer may affect the dissolution of the dispersion to a greater degree than the presence of a small molecule excipient would. Polyvinylpyrrolidone (PVP) has been used on a number of occasions to create dispersions of a variety of drugs, including indomethacin, the model drug used in this chapter. Acrylic acid polymers were selected to formulate the tertiary dispersions as they have been shown to interact with PVP. This interaction is between the hydroxyl groups of poly(acrylic acid) (PAA) and the carbonyl groups of PVP (Florence and Attwood, 1998).

Indomethacin is considered to be relatively miscible with PVP and it also displays a strong interaction with PVP thus the target molecular dispersions should be relatively easy to produce. The strong interaction should also ensure that the amorphous indomethacin is stable for the duration of testing (Taylor and Zografi, 1997; Matsumoto and Zografi, 1999; Watanabe *et al.*, 2003; Yoshioka *et al.*, 1995). Acrylic acid polymers display a wide range of glass transition temperatures (T_g) and solubility properties. They are also widely used in pharmaceutical as film-coating agents.

Solid indomethacin can exist in a number of polymorphic forms. The stable γ-crystal polymorphic form and the metastable α-crystal polymorphic form are the most common. Amorphous indomethacin is itself relatively instable and begins to crystallise within a few weeks (Andronis and Zografi, 2000), though stability has been improved by producing dispersions in which indomethacin was the major component and PVP a minor component. The effect was observed when PVP made up only 5% of the mass of the dispersion. At this concentration of PVP, the T_g was less than 50 °C above the storage temperature. 50 °C is considered to be the critical temperature for the minimisation of molecular motion and inhibition of crystallisation (Hancock *et al.*, 1995; Matsumoto and Zografi, 1999).

The stabilising effect was ascribed to a specific interaction between the hydroxyl section of the carboxylic acid group of indomethacin, which is highlighted in red in Figure 3.1, and the carbonyl group of PVP. This prevents recrystallisation as the indomethacin diamers are believed to be the base unit of the crystals (Taylor and Zografi, 1997; Matsumoto and Zografi, 1999). As a specific interaction is seen the amorphous stability

shown by indomethacin when formulated with PVP is therefore much greater than expected. It is important to note that the level at which they observed an improvement in stability occurred well below the 1:1 molar ratio one would expect.

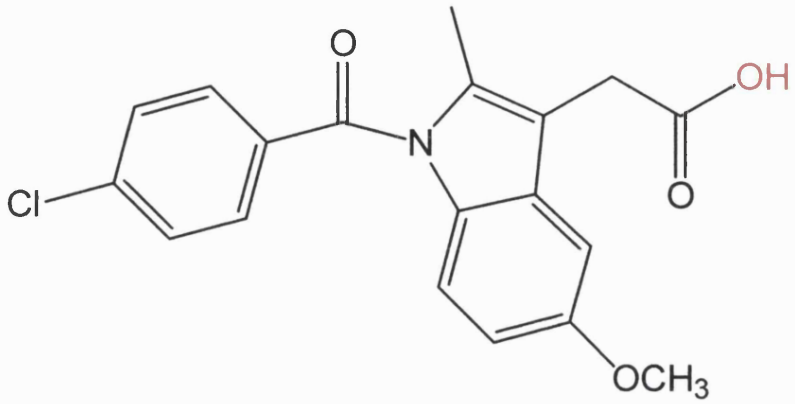

Figure 3.1 The structure of indomethacin with the hydroxyl group highlighted in red.

A good literature example of a tertiary system that has been examined is that of probucol-PVP-PAA tertiary dispersion. These dispersions have been produced and compared with probucol-PVP binary dispersions. The tertiary system was composed of probucol 50% w/w, PVP 25% w/w and PAA 25% w/w, whilst the binary system consisted of probucol 50% w/w and PVP 50% w/w. The researchers concluded that the replacement of some PVP with PAA was in that particular case detrimental to the amorphous stability probucol. The binary dispersion was found to be completely amorphous whilst the tertiary system showed a minor melt event, though this was not quantified (Broman *et al.*, 2001). The presence of crystalline material is to a degree unexpected; probucol (the structure of which is shown in Figure 3.2) is able to interact with both PVP and PAA through its hydroxyl group. Additionally PVP and PAA display a strong interaction which can result in a combined T_g that is in excess of the T_g of the constituents (Lau and Mi, 2002 Florence and Attwood 1998).

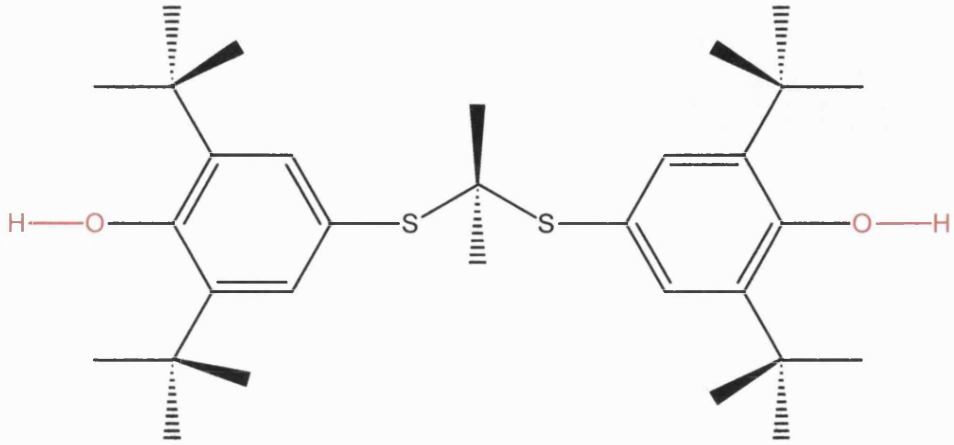

Figure 3.2 The structure of probucol with the hydroxyl groups highlighted in red.

3.1.1 Preparation of Solutions

In order to produce homogenous solid dispersion microparticles, one would expect that the feed solution should also be homogenous. Should particles be present in the solution one would expect the solid dispersion microparticles to lack homogeneity. Similarly parts of the feed solution rich in a particular component would be expected to result in some particles rich in the same component. The dispersions will consist of a drug with poor water solubility combined with relatively water soluble polymers which may be poorly soluble in organic solvents. These solvents may also prove to immiscible with water. Therefore one has to create a single phase solvent system that is capable of dissolving all the components of a dispersion. Specific interactions between the various constituents may also occur in solution which could lead to precipitation. Hence one must not only look at the solubility of components in isolation, one must examine how the presence of one component may affect the dissolution of further components.

3.1.2 Solvent Choices for Dissolution Studies

Initially two main solvents were selected for spray drying; ethanol and water. Both the Food and Drug Administration and the European Agency for the Evaluation of Medicinal Products consider these to be safe (Class 3), therefore there are no absolute limits on the residual levels of these solvents provided the levels in a finished product can be justified. These solvents are also miscible with one another, hence combinations of the two solvents could be considered.

3.1.3 The Effect of a Second Polymeric Excipient on the Release of Indomethacin From Indomethacin and Polyvinylpyrrolidone Solid Dispersions

As was stated in Section 3.1 the dissolution rate of drugs from solid dispersions is often related to the dissolution rate of the polymer that makes up the bulk of the dispersion. It was decided that an investigation into the use of secondary excipients would be performed to find out how they would affect dissolution behaviour. As acrylic polymers had been selected as the secondary polymeric excipient, one of the first areas of interest was to be the effect of pH on the dissolution rate of indomethacin from the polymer bulk.

Indomethacin has been shown to be stable in PVP and the mechanism by which this stabilisation takes place is reasonably well understood. The system therefore makes a good control group against which tertiary dispersions (or dispersions containing a second excipient) can be tested. As was discussed in Section 1.8 crystalline material is generally less soluble than amorphous material (Hancock and Parks, 2000; Shekunov and York, 2000). The concentrations of indomethacin and of the secondary polymeric excipient were therefore kept purposefully low in order to minimise the possibility that the indomethacin might crystallise in the tertiary dispersions. Having a low concentration of indomethacin should also mean that the dissolution studies would take place in sink conditions; where sink conditions are a maximum concentration of 10% of the saturation solubility of the chemical moiety in question (Aulton, 2001).

3.2 Methods

3.2.1 Drug Dissolution Experiments

Indomethacin is sparingly soluble in ethanol which means that between 10 and 100 ml of ethanol is required to dissolve 1 g of indomethacin (British Pharmacopoeia, 2005b). The entry for indomethacin in "Analytical Profiles of Drug Substances" states that the solubility of indomethacin in distilled water is 40 μg/ml, however in pH 7 phosphate buffer the solubility is an order of magnitude higher (O'Brien *et al.*, 1984). This is shown in Table 3.1. To better define indomethacin solubility γ-indomethacin (100 mg) was added to stirred ethanol (1 ml) in a 2 ml vial and dissolution allowed to proceed at 30 °C for 24 h. The supernatant was then filtered through a 0.45 μm filter and diluted to 20 ml. The absorbance of the indomethacin at 318 nm was taken and the concentration of indomethacin in solution was then calculated against a calibration curve consisting of

5 data points produced by taking the average absorbance of 3 readings at indomethacin concentrations of 0.001, 0.002, 0.003 and 0.004 mg/ml. The effect of the addition of water on a solution of indomethacin in ethanol was then examined. Indomethacin (160 mg) was dissolved in stirred ethanol (4 ml) in an 8 ml vial. Distilled water was then added in 200 µl quantities at intervals of 15 min until precipitation was seen.

Phosphate buffer pH	Solubility (µg/ml)
5.6	30
6.2	110
7.0	540

Table 3.1 The solubility of indomethacin in phosphate buffer (O'Brien et al., 1984).

3.2.2 Polymer Dissolution Experiments

Each polymer (1 g) was in turn accurately weighed into a 14 ml glass vial into which 10 ml of the required solvent, either water or ethanol (at 20 °C), was then added. The vial was then sealed and shaken by hand for around 10 min. If the polymer did not dissolve a magnetic flea was then added and the solution stirred for 6 h. Dissolution was then checked every 30 min.

The various acrylate polymer solutions were in turn combined with a PVP solution. The PVP solution (1 ml, 10% w/v) was transferred to a 2 ml glass vial which was sealed with a rubber septum. Polyacrylate solution (100 µl, 10% w/v) was slowly added and the vial was gently shaken by hand. Analysis of the dissolution was by visual inspection against a dark background as none of the excipients contained a chromophore. Should any precipitation be seen this was noted and a second experiment was performed. A stirred solution of PVP (1 ml, 10% w/v) in a 2 ml glass vial sealed with a rubber septum was prepared. The polyacrylate solution was then slowly injected into the stirring PVP. The solution was stirred for a further 2 h and was then visually inspected for the presence of precipitates. Further details of these and other experiments are presented in Section 2.2.

3.2.3 The Addition of a Solution of PAA to a Solution of PVP

A solution of poly(acrylic acid) (PAA; M_w 250 000) in water (10 ml, 10% w/v) was prepared in a 10 ml vial using the technique in Section 3.2.2. This was poured slowly into a stirred solution of PVP K30 (M_w 50 000) in water (10 ml, 10% w/v) which had

been prepared in a 25 ml round bottomed flask. Any precipitation was to be removed by filtration using a Buchner funnel. The filter cake was dried for 48 h under vacuum at room temperature. Infrared (IR) spectroscopy was then used to identify the components that had precipitated. Differential scanning calorimetry (DSC) was used to locate the T_g of the sample. The precipitate (1.5-8 mg) was loaded into a DSC pan and was heated to 100 °C at a rate of 10 °C/min. The sample was held at this temperature for 5 min before being cooled to 20 °C and then heated to 250 °C at 10 °C/min. This was repeated with 3 further samples and an average T_g was taken. The T_g of each of the components was then located using the same method.

3.2.4 PREPARATION OF INDOMETHACIN DISPERSIONS

A control dispersion of indomethacin and PVP was prepared by the following method. Indomethacin (1 g) was accurately weighed and dissolved in stirred ethanol (80 ml) in a 250 ml conical flask. Distilled water (20 ml) was then added and the solution was allowed to mix for a further 20 min. PVP (10 g) was accurately weighed and slowly poured into the stirred solution. The mixture was stirred for 20 min. The resultant solution was spray dried using the SD Niro system with inlet and outlet temperatures of 70 °C and 45 °C respectively. The nitrogen flow rates were 2 kg/h for atomisation and 20 kg/h for drying. The flow rate for the feed solution pump to the nozzle was 20% of the maximum rate which equates to a flow rate of the feed solution of approximately 3 ml/min.

Tertiary dispersions containing indomethacin, PVP and a second excipient were also prepared in a similar fashion to that described above. Their preparation is described in the following sections. The polymers selected for the dispersions were as follows; PAA M_w 2000, poly(2-hydroxypropylmethacrylate) (PHPMA) M_w 20 000, Eudragit L and Eudragit S. All were chosen as they could interact with both indomethacin and PVP via hydrogen bonding. The target ratio for indomethacin to PVP to the second excipient was 1:8:2 respectively. A low concentration of indomethacin was utilised in order to prevent any difficulties with producing sink conditions in the dissolution testing which might alter dissolution. Furthermore the use of a low drug concentration would minimise the risk of indomethacin crystallisation. The low level of secondary polymeric excipient was selected to prevent any risk of indomethacin crystallisation due

to competition for carbonyl groups on the PVP. The order in which the various constituents of the dispersion were dissolved is shown in Table 3.2.

	Dispersion Order Number		
Component	**1**	**2**	**3**
1	2nd Polymer	Indomethacin	Indomethacin
2	PVP	PVP	2nd Polymer
3	Indomethacin	2nd Polymer	PVP

Table 3.2 The order in which the components of the dispersions were dissolved.

3.2.5 "ORDER ONE" INDOMETHACIN DISPERSIONS

The secondary polymer (2 g) was weighed and dissolved in stirred ethanol (80 ml) in a 250 ml conical flask. Distilled water (20 ml) was poured slowly into the stirred solution. PVP (8 g) was added and the solution was allowed to mix for a further 2 h. The solution was then checked for precipitates. Should solid polymer still be visible then further ethanol (10 ml) was added followed by distilled water (15 ml) and the solution stirred for 1 h. Having dissolved the polymers indomethacin powder (1 g) was slowly added and allowed to dissolve over 2 h. The resulting solution was spray dried as described for the control groups.

3.2.6 "ORDER TWO" INDOMETHACIN DISPERSIONS

Indomethacin (1 g) was dissolved in stirred ethanol (80 ml) in a 250 ml conical flask. PVP (8 g) was slowly poured into the stirred solution and allowed to dissolve for 15 min. Distilled water (20 ml) was added and the solution allowed to mix. After 15 min of mixing the secondary polymeric excipient (2 g) was added and the solution was stirred for a further 2 h. The solution was then checked for precipitates. If any solid material was detected then ethanol (10 ml) and distilled water (10 ml) were added and the solution was allowed to mix for a further 2 h before being spray dried.

3.2.7 "ORDER THREE" INDOMETHACIN DISPERSIONS

Indomethacin (1 g) was dissolved in a stirred solution of ethanol (80 ml). After 15 min distilled water (20 ml) was added. After a further 15 min the second excipient (2 g) was added and the solution stirred for a further 2 h. Following this ethanol (10 ml) and of distilled water (10 ml) were added and the solution allowed to mix for 15 min. PVP (8

g) was added and the solution stirred for a further 2 h. The resultant solution was then spray dried.

3.2.8 Analysis of Solid Dispersions

The spray dried samples were stored under continuous vacuum for 1 week at ambient temperature to remove residual solvent. The dispersions were analysed by thermo-gravimetric analysis (TGA) between 30-150 °C, differential scanning calorimetry (DSC) between 30-200 °C and X-ray powder diffraction (XRPD). Dissolution studies were then performed in 0.1 N HCl (pH ≈ 1.2), pH 6.5 phosphate buffer solution and pH 7.0 phosphate buffer solution.

Dispersion microparticles (10-20 mg) were loaded into clear hard gelatine capsules (size 1). Dissolution studies were then performed on four such capsules containing each of the dispersions in 0.1 N HCl (pH ≈ 1.2), pH 6.5 phosphate buffer solution or pH 7.0 phosphate buffer solution (900 ml). 13 samples (including an initial sample) were taken over the following 45 min. Each of the dispersions containing a second excipient was compared with a control dispersion produced at the same time that only contained indomethacin and PVP. The absorbance of the indomethacin within the samples of the dissolution media at the literature value for the λ_{max} of indomethacin (318 nm) was taken (O'Brien *et al.*, 1984). The concentration of indomethacin in the dissolution media was then calculated using the least squares regression of a calibration curve. The calibration curve consisted of 4 data points.

3.2.9 Preparation of the Calibration Curves

The approximate indomethacin concentrations used to prepare the calibration curve data points were 0.001, 0.002, 0.003 and 0.004 mg/ml. Each of the data points was produced by taking an average of 3 UV readings of indomethacin absorption which had been dissolved in the relevant dissolution media (0.1 N HCl (pH ≈ 1.2), pH 6.5 phosphate buffer solution or pH 7.0 phosphate buffer solution). Indomethacin (100-400 mg) was accurately weighed in a Class A, 20 ml volumetric flask. Ethanol was added to make the volume up to 20 ml. The volumetric flask was then allowed to mix for 12 h on a Baird and Tatlock Rotor Mixer at a speed of approximately 60 rpm. A quantity of the resultant solution (200 µl) was transferred to a Class A, 1 L volumetric flask using a 200 µl Gilson Pipetman. This was then made up to volume with the dissolution media. A magnetic stirrer bar was added and the solution was allowed to mix for a further 12 h.

UV analysis of the resultant solutions was performed using a Cary 3E UV-Vis spectrophotometer connected to a PC running Cary WinUV v3.0 (both Varian, Inc., Palo Alto, USA). Each sample was taken from dissolution test apparatus and transferred to one of a matched pair of quartz cuvettes (10-mm path length). The other cuvette was used as a blank and was filled with the dissolution media. The UV absorption at 318 nm was taken. The "least squares" linear regression line of the calibration curve calculated using the "LINEST" function in Microsoft® Office Excel 2003 was then used to calculate the concentration of the solution. This value was then used to calculate the percentage of indomethacin that had dissolved. A worked example is presented in Section 3.3.4.

3.3 Results and Discussion

3.3.1 Indomethacin Solubility in Ethanol

The limit of solubility for indomethacin in ethanol at room temperature was found to be 42 mg/ml. This showed that it would be possible to produce spray dryer feed solutions at an acceptable concentration. As expected the solubility of indomethacin in distilled water was found to be very low with a saturation solubility of 36 µl/ml. When testing the effect of water on ethanolic solutions of indomethacin a total 1 ml of water was added to the indomethacin solution in ethanol (160 mg in 4 ml) before precipitation was seen. Therefore it was shown to be possible to produce feed solutions of indomethacin in a mixed solvent.

3.3.2 Polymer Solubility

The Eudragit product summary states that Eudragit L and S are known to be soluble in alcohols including ethanol up to a 12% concentration of polymer and in acetone (containing 3% water) to a comparable concentration (Degussa, 2004). The distilled water used in all sections of this chapter was slightly acidic with a pH of approximately 5.9. Eudragit L is known to be insoluble below pH 6.5, whilst Eudragit S is known to be insoluble below pH 7.0. As expected, neither polymer was found to be soluble in the distilled water. PAA M_w 2 000 and 250 000 were both found to be soluble to at least 100 mg/ml in both ethanol and water after being stirred for 8 h. Poly(2-hydroxypropylmethacrylate) (PHPMA) M_w 20 000 was also found to be soluble in distilled water however it required 2 h to dissolve completely. PHPMA was found to be

insoluble in ethanol. Polyvinylpyrrolidone (PVP) was found to be rapidly soluble in both ethanol and water, to at least 100 mg/ml taking around 10-15 min to dissolve.

3.3.3 THE COMBINATION OF PVP AND PAA SOLUTIONS

As a PAA in water (10% w/v) solution was slowly added to a stirred PVP solution in water (10% w/v) a large mass of polymer was seen to precipitate out of solution. The supernatant was decanted and the damp mass of polymer placed in a glass bottle. Following a drying period of 1 week at room temperature under continuous vacuum, the sample was found to be difficult to grind using a pestle and mortar and was insoluble in distilled water, ethanol and mixtures of the two solvents. Analysis by DSC using a scan rate of 10 °C/min as described in Section 2.5.1 indicated that the onset T_g was 214.5 (± 0.5 °C). This was in good agreement with the T_g previously stated for PVP-PAA complexes which was shown to be 205 °C (Lau and Mi, 2002). Analysis by IR of the sample showed a slight shift in the wavelength of the carbonyl peak (Figure 3.3), which was indicative of an interaction between these groups and the hydroxyl groups of PAA (Lau and Mi, 2002). A similar precipitate was seen when the experiment with PVP and PAA was repeated using ethanol as the solvent for each sample. It was concluded that it would prove to be difficult to produce dispersions which contained PVP and PAA M_w 250 000. Additionally there were concerns that similar precipitation could occur with any of the second excipients being considered for the preparation of dispersions. This would make it difficult to produce a spray dryer feed solution. Even if spray drying proved to be possible concerns remained that the dissolution rate might be reduced as in this case it was not possible to redissolve the mass that had precipitated.

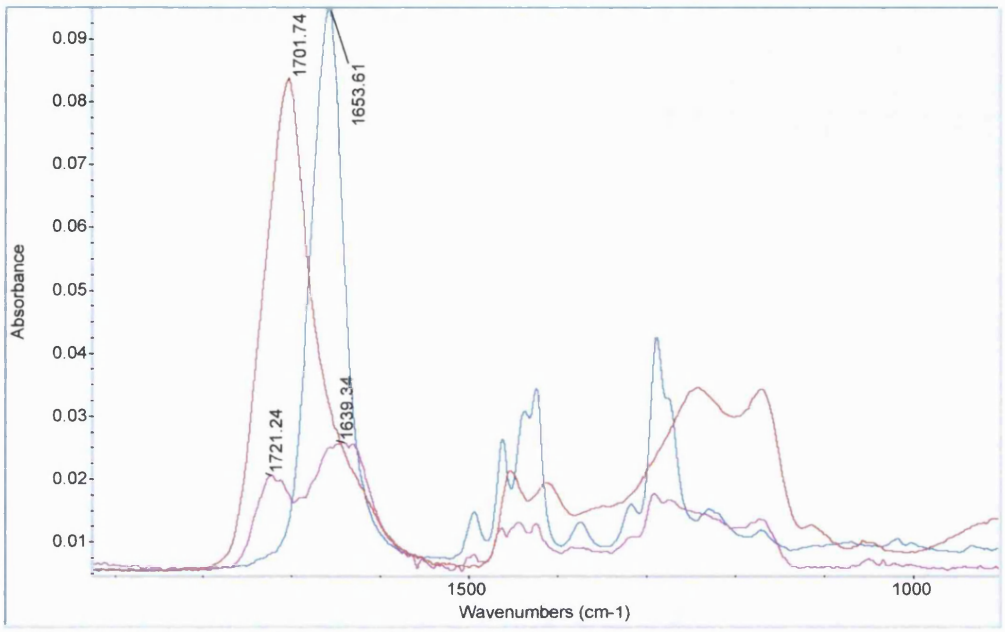

Figure 3.3 The absorbance of PAA (red), PVP (blue) and PAA-PVP complex (purple) between 950-1900 cm^{-1} taken by Fourier transform infrared (FTIR).

When 10% solutions of PVP K50 and PAA M_w 2000 in ethanol were added together a similar level of precipitation was seen, however the precipitate consisted of a number of discrete particles rather than a single mass and the addition of a small amount of water appeared to reverse the precipitation, with the components redissolving in an ethanol and water mixture. When the experiment was repeated with 10% solutions of PVP in water and PAA M_w 2000 in water, the solution appeared to be homogenous. It was concluded that precipitation was dependent on chain length.

When the experiment was repeated with a solution of Eudragit L in ethanol (10% w/v) which was then added to a solution of PVP in water (10% w/v) there was no precipitation. However when Eudragit L in ethanol was added to PVP in ethanol a small amount of precipitation was seen although again this proved to be reversible on the addition of distilled water.

It was concluded that the presence of water would permit a degree of ionisation in the PAA, Eudragit L or Eudragit S, thus reducing the level of interaction between these polymers and PVP as hydrogen-bonding would be reduced. As the small scale solubility studies had suggested that indomethacin was soluble in ethanol containing a maximum of around 20% v/v water a combination of solvents had been found that permitted the dissolution of all three components in a variety of orders.

3.3.4 INDOMETHACIN DISPERSIONS SPRAY DRIED FROM SOLUTIONS PREPARED USING ORDER ONE; RESULTS AND DISCUSSION

When analysed by XRPD, all of Order One was found to be completely amorphous as was to be expected due to the aforementioned interaction. Analysis by TGA at 10 °C/min between 30-180 °C gave the percentage weight loss. This weight loss was ascribed to residual solvent as the sample did not change colour, char or blacken in the TGA across this temperature range. Furthermore the DSC results showed a smooth peak across the range 50-100 °C. As the amount of physical degradation was expected to increase as temperature increased and degradation is usually observed as sharp spikes of noise an assumption was made that the weight loss was due loss of residual solvent. The values are shown in Table 3.3. Analysis by DSC at 10 °C/min between 30 °C and 200 °C provided the T_g of each of the dispersions (Table 3.3). The results of the DSC analyses showed the amorphous nature of indomethacin in all of the dispersions as the indomethacin melt was not detected in any of the samples.

Dispersion content	Percentage residual solvent	T_g (°C)
Indomethacin and PVP	2.1 ± 0.2	151.0 ± 1.3
Indomethacin, PVP and PHPMA	2.3 ± 0.2	142.3 ± 0.6
Indomethacin, PVP and PAA	2.0 ± 0.0	141.1 ± 0.2
Indomethacin, PVP and Eudragit L	1.7 ± 0.4	157.7 ± 0.4
Indomethacin, PVP and Eudragit S	1.6 ± 0.1	172.5 ± 1.0

Table 3.3 Residual moisture content and the T_g for dispersions of indomethacin made as per "Order One".

Residual solvent values as determined by TGA were within the range 1.5%-2.3%. This solvent loss was seen between 50-120 °C with the rate of weight loss being relatively consistent across this range. As the solvent loss occurred over such a large temperature range it was difficult to ascribe it to any particular proportion of water and ethanol. Furthermore the nature of spray drying means that the solvents were most likely

entrapped in the centre of the particles as drying is thought to produce an outer crust on the particle through which the solvent held in the centre of the particle diffuses (Farid, 2003). This could produce a lag time between the heating of the air around the particle which is detected by the TGA thermo-couple and the temperature at the particle core.

DSC results indicated that all these dispersions had a T_g of 140-160 °C with the exception of the indomethacin, PVP and Eudragit S dispersion which had a T_g of 172.5 °C. Given that Eudragit S contains fewer carboxylic acid groups than either Eudragit L or PAA this is unlikely to be due to an increased level of interaction and is therefore most likely to be due to the intrinsic properties of the polymers. Whilst the indomethacin, PVP and Eudragit S dispersion did contain less residual solvent which may have had an effect, this effect was not considered sufficient to account for such a large difference in T_g. According to Degussa the T_g of both Eudragit L and Eudragit S is approximately 160 °C. They have also examined the plasticizing effect of PEG on the T_g. When PEG was dispersed in the polymers (10% w/w), the T_g of Eudragit L falls to 115 °C whereas the T_g of Eudragit falls to 100 °C (Degussa, 2002). One would therefore expect to see the dispersion containing Eudragit L to have a slightly higher T_g. This did not prove to be the case. The T_g of PVP was found experimentally to be approximately 168 °C, which is remarkably close to that of the dispersion. Therefore it may be that the dispersion containing Eudragit S did have two separate phases. However this could not be confirmed by hyper DSC. Indeed, heat-cool-heat experiments showed that the Eudragit S dispersion had a consistently high T_g where one might expect the T_g to fall as the constituents dissolved in each other on each heating step.

Dissolution studies were performed in 0.1 N HCl (pH ≈ 1.2), pH 6.5 phosphate buffer solution and pH 7.0 phosphate buffer solution. The percentage of indomethacin that had dissolved was calculated on the basis of the absorbance at 318 nm. This was compared with a calibration curve produced using the same media. Prior to discussing the results of the dissolution study a worked example of the calculations is provided.

A sample absorbance reading for the final data point of one of the capsules containing the indomethacin, PVP and PAA dispersion from the dissolution study in phosphate buffer solution pH 6.5 (Figure 3.6a; page 84) was found to be 0.0503. This is converted

into a concentration in mg/L using a conversion factor taken from the line of best fit produced using the "LINEST" function in Microsoft® Office Excel 2003 from a calibration curve. The approximate indomethacin concentrations used to prepare the calibration curve were 1, 2, 3 and 4 mg/L. The actual concentration values used and the absorbance for each concentration are shown in Table 3.4. The calibration curve including the value produced by the LINEST equation is shown in Figure 3.4 and it was found to be 0.0196. It therefore follows that:

$$0.503/0.0196 = 2.57 \text{ mg/L}$$

Concentration (mg/L)	Average Absorbance
1.05	0.0216
2.10	0.0423
3.15	0.062
4.20	0.081

Table 3.4 The absorbance at 318 nm of solutions of known concentrations of indomethacin in phosphate buffer solution pH 6.5

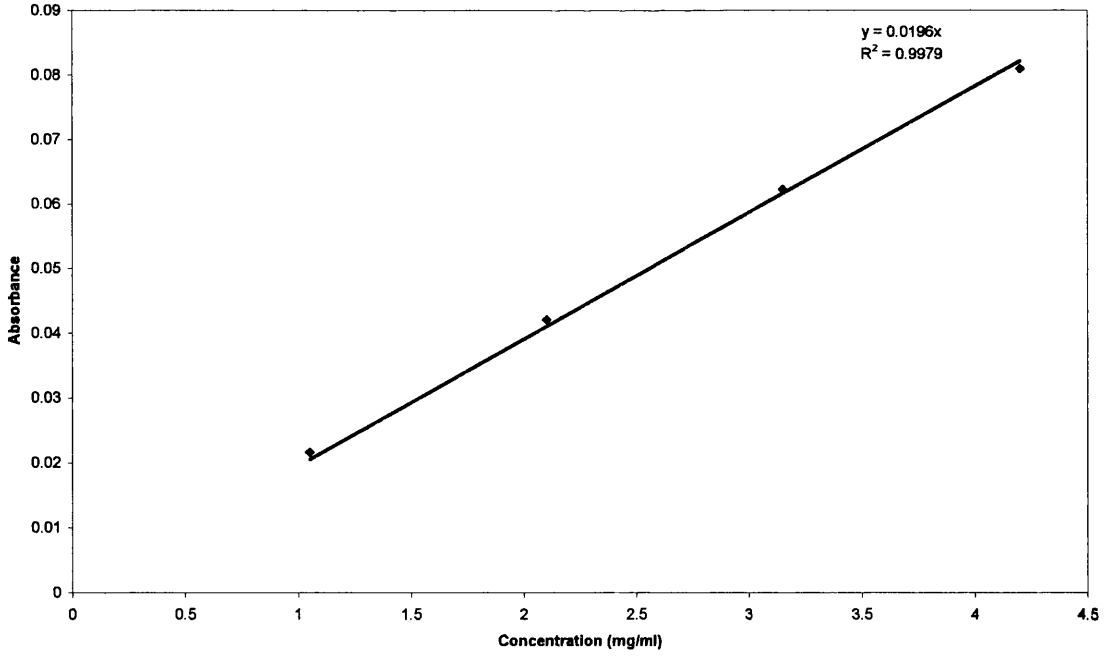

Figure 3.4 A calibration curve showing the absorbance of solutions of indomethacin. A least squares linear regression line has been marked on the graph as has the equation for this line and the R^2 value.

Having found that the concentration of indomethacin was 2.57 mg/L the next step was to turn this into the total amount of griseofulvin dissolved. As this value was taken at the end of the dissolution study 36 ml of dissolution media had been removed. It therefore follows that:

$$2.57/1000*(900-36) = 2.22 \text{ mg}$$

So 2.22 mg of indomethacin had dissolved by the end of the dissolution study from an indomethacin, PVP and PAA dispersion in which indomethacin made up an eleventh of the total weight. The original weight that was loaded into the capsule prior to the dissolution study was 24.56 mg.

$$24.56/11 = 2.23 \text{ mg}$$
$$100/2.23*2.22 = 99.55\%$$

Therefore, for this sample 99.55% of the indomethacin that was theoretically present in the dispersion actually dissolved.

Figure 3.5 shows the results of the dissolution study performed in 0.1 N HCl. Indomethacin is a weakly acidic drug. At low pH indomethacin is very poorly water soluble as it does not become ionised. This meant that sink conditions were not achieved in 0.1 N HCl. Despite this, the dispersions containing PAA, Eudragit L and Eudragit S displayed significantly less dissolution (Student's *t*-test; $p > 0.05$) than the binary indomethacin and PVP dispersion which was being used as a control and the indomethacin, PVP and PHPMA dispersion. PAA and both Eudragit L and S are themselves at least weakly acidic and therefore do not dissolve at low pH.

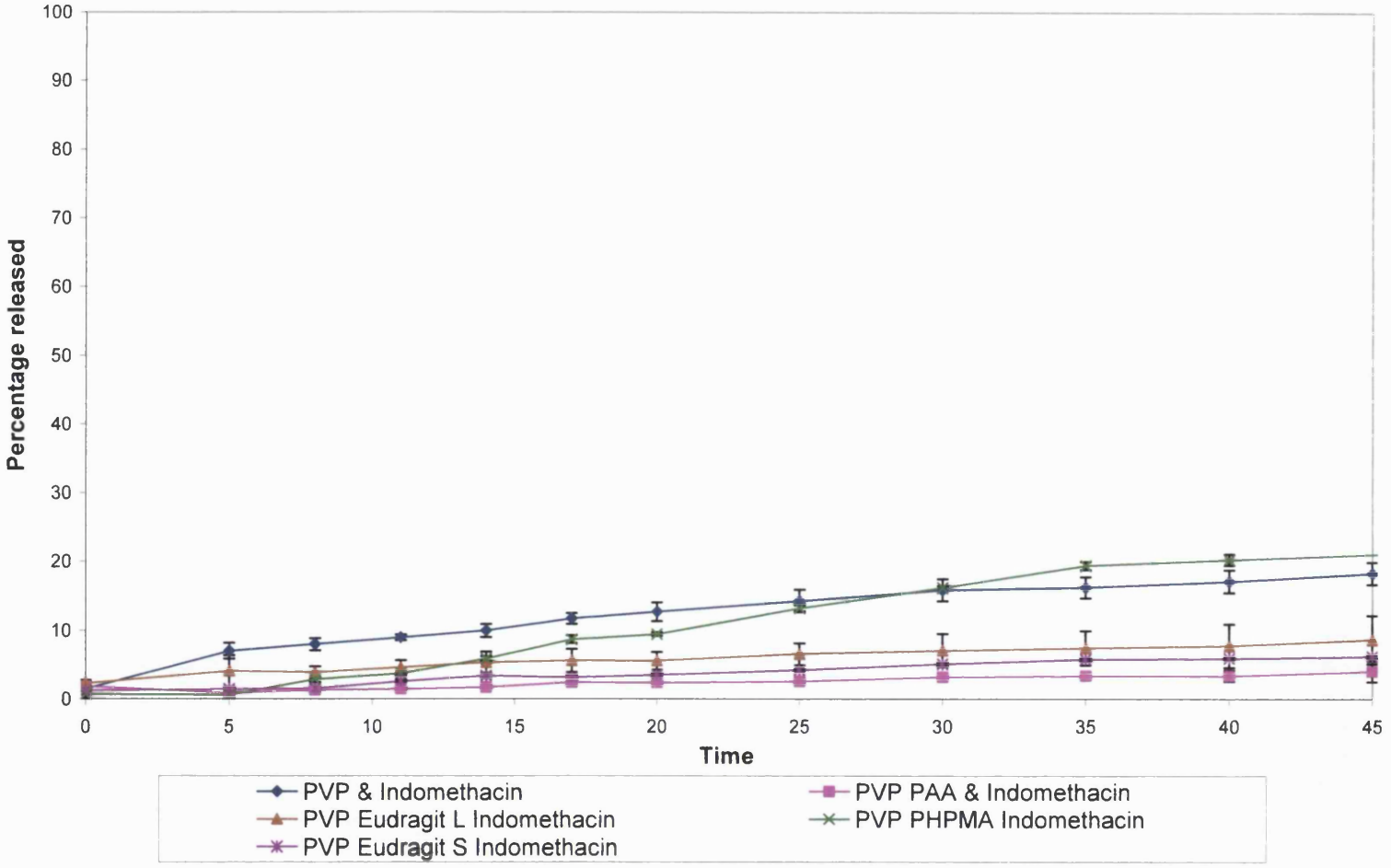

Figure 3.5 The dissolution of indomethacin from spray dried microparticles contained within hard gelatin capsules into 0.1 N HCl over time expressed as percentage indomethacin released.

At pH 6.5 (Figure 3.6a) near complete indomethacin dissolution occurred within 45 min from all the dispersions including the indomethacin and PVP binary dispersion which acted as the control. The one exception was the indomethacin, PVP and Eudragit S dispersion which displayed little dissolution over the course of the dissolution study.

A further experiment with this formulation was performed to determine the extent of dissolution over 5 h. Again (Figure 3.6b) there was still no indomethacin dissolution. This was surprising given that Eudragit S made up less than 20% w/w of the dispersion and the main component, PVP, is known to be highly water soluble across a range of pH. The indomethacin, PVP and PAA dispersion displayed a pseudo-zero order release profile. In a related study by Chun and co-workers (2002) a similar effect was observed in tablets of ketoprofen made with PVP and PAA. The tablets were produced by dissolving PVP and acrylic acid in ethanol. The acrylic acid was then polymerised to produce PVP-PAA complexes. The dried complex was compressed with ketoconazole.

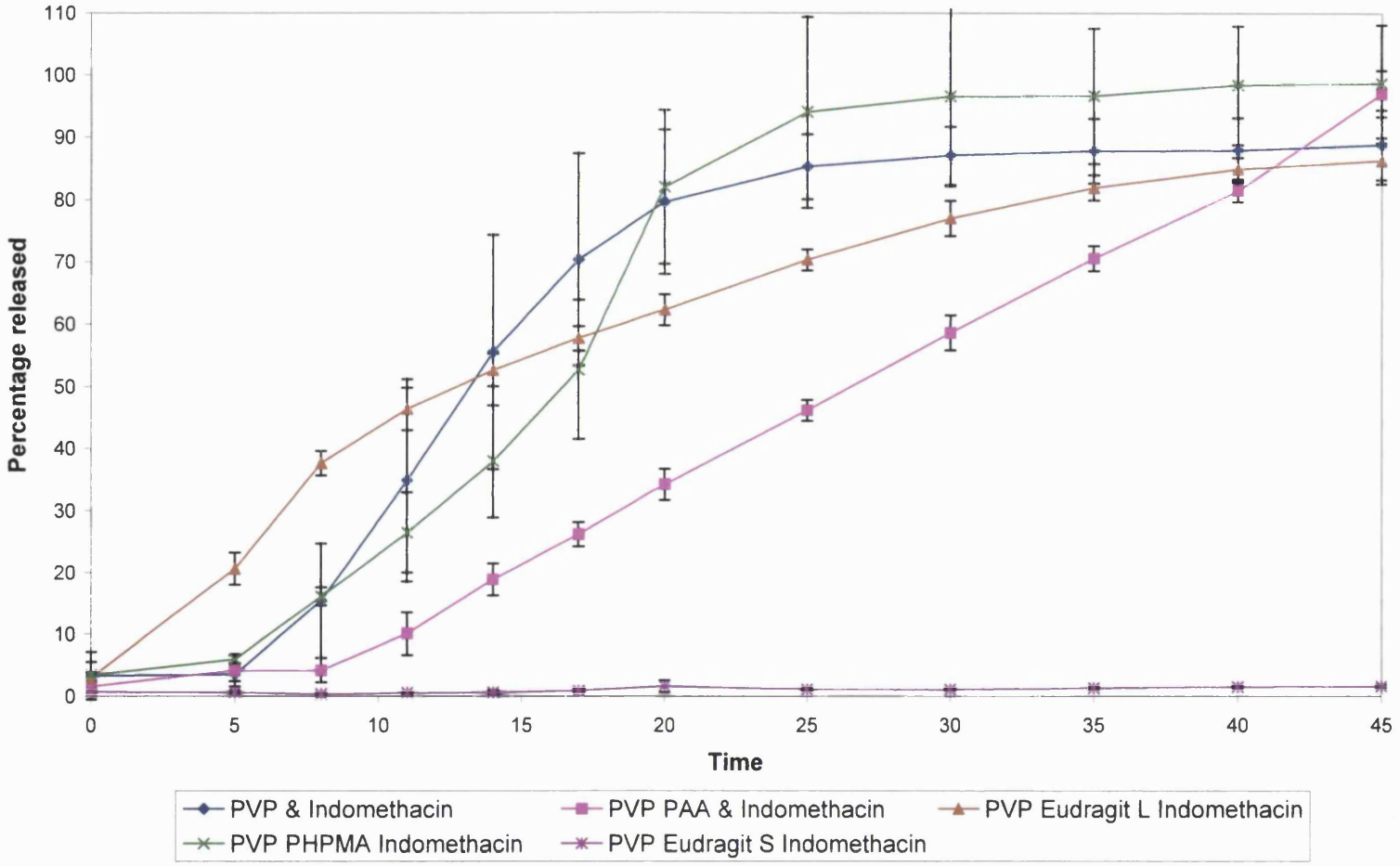

Figure 3.6a The dissolution of indomethacin from spray dried microparticles contained within hard gelatin capsules into pH 6.5 buffer over time expressed as percentage indomethacin released.

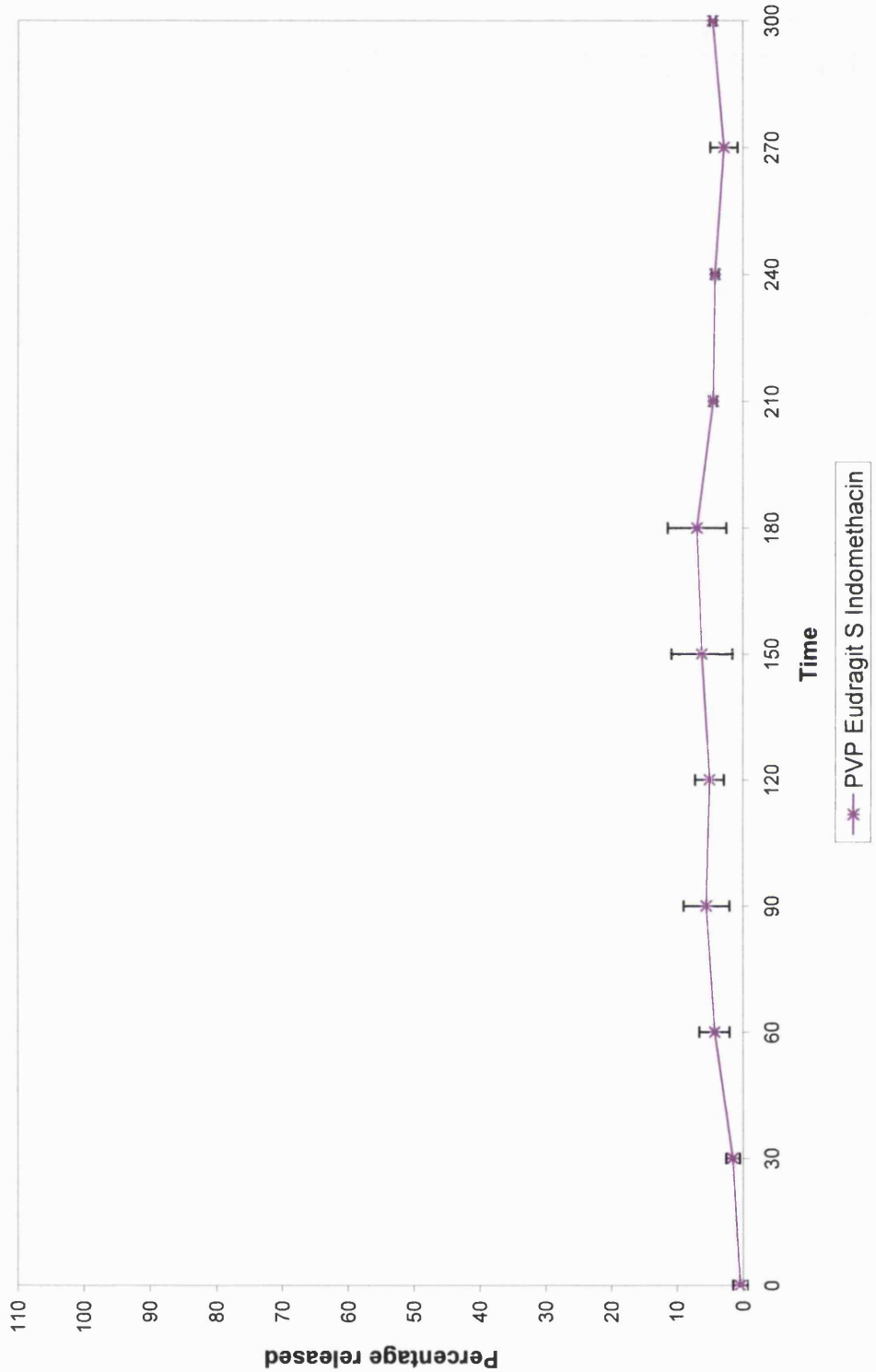

Figure 3.6b The dissolution of indomethacin from spray dried microparticles containing PVP and Eudragit S of within hard gelatin capsules into pH 6.5 buffer over 300 min expressed as percentage indomethacin released.

At pH 7.0 complete indomethacin dissolution was seen from all the dispersions prepared by the method in Section 3.2.5 (Order One) including the indomethacin, PVP and Eudragit S dispersion (Figure 3.7) as was expected. Indomethacin from this dispersion failed to dissolve at pH 6.5. Eudragit S is insoluble below pH ≈ 6.9-7.0 and thus is used to target the colon (Van den Mooter, 2006; Degussa, 2002). It is likely that the intrinsic dissolution properties of the secondary polymeric excipient had an effect on indomethacin dissolution from the dispersion. This will be discussed further below. Once again a zero order release profile was observed for the indomethacin, PVP and PAA dispersion, suggesting that dissolution was again controlled by the minor excipient.

Given that the secondary polymeric excipient makes up less than 20% of the weight of the dispersion whilst the drug only makes up less than 10% it is difficult to understand exactly why the dispersion would function in this way. Hypothetically, it is possible that there were two distinct phases in the dispersions as discussed above. The first would consist of Eudragit S and indomethacin (with perhaps some PVP) and the second would consist of PVP. However one would expect two separate glass transitions to have been detected during the DSC experiments; a glass transition at a lower temperature corresponding to a complex of the secondary polymer and indomethacin and a second glass transition at a higher temperature corresponding to that of the pure PVP. As stated in Table 3.3 a single glass transition was observed at 172.5 °C ±1.0 °C.

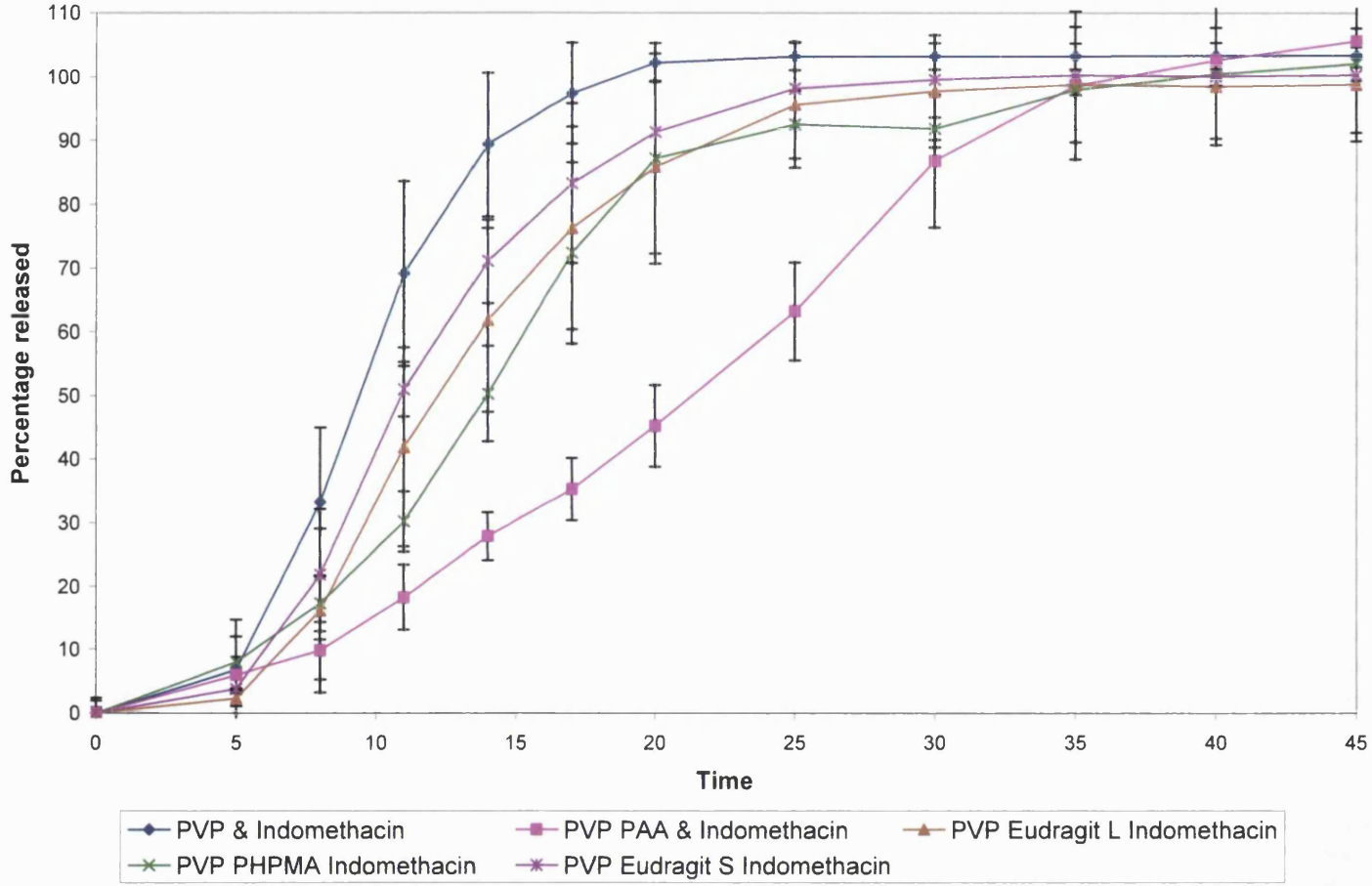

Figure 3.7 The percentage dissolution of indomethacin from spray dried microparticles contained within hard gelatin capsules into pH 7.0 buffer over time expressed as percentage indomethacin released.

3.3.5 ORDER TWO RESULTS AND DISCUSSION

The dispersions produced using Order Two differ from Order One solely by virtue of the order in which the various components were dissolved prior to spray drying. The control group was again the indomethacin and PVP dispersion. Following the results from Batch One the decision was made to focus on the dissolution of the indomethacin, PVP and Eudragit S dispersion at pH 6.5 and pH 7 and the indomethacin, PVP and PAA dispersion as the dispersions had both shown differences in dissolution properties from the indomethacin and PVP dispersion. An attempt was made to create an indomethacin, PVP and Eudragit L dispersion, however it was not possible to dissolve fully all the components in the spray dryer feed solution and the feed solution had a milky opaque character. As with Order One dispersions, both XRPD and DSC confirmed the amorphous nature of the dispersions. Figures 3.8 and 3.9 show the raw XRPD and DSC data for the indomethacin and PVP dispersion which was the control group whilst Figures 3.10 to 3.13 show the raw XRPD and DSC data for the two dispersions produced using Order Two. As can be seen neither the level of residual solvent nor the T_g significantly differed from those seen in the Order One dispersions (Table 3.5).

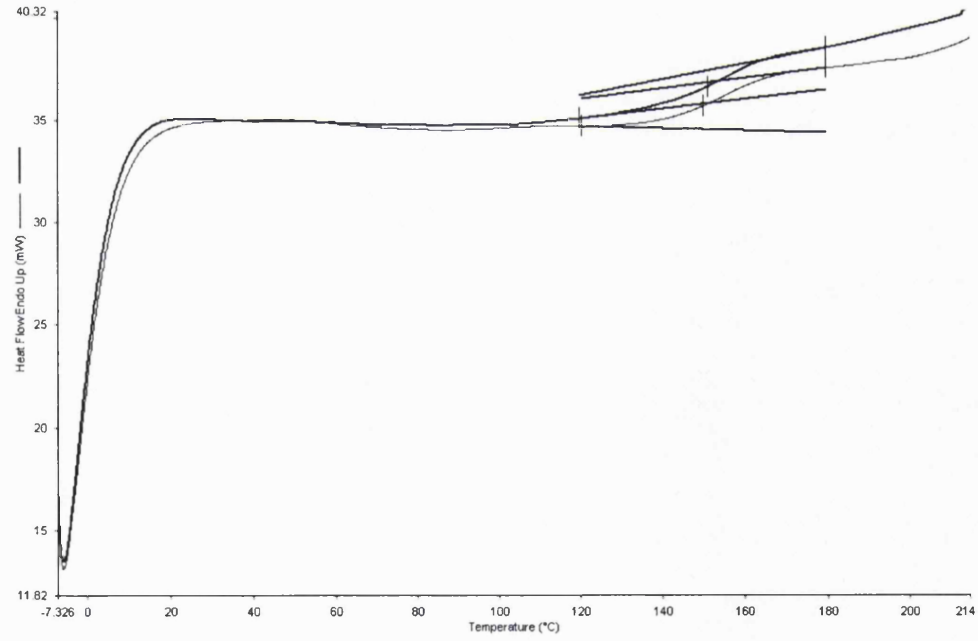

Figure 3.8 A sample DSC trace of an indomethacin and PVP dispersion showing the T_g at around 150 °C. The bold line is the original trace, and the faint line is an adjusted trace, flattened to improve the accuracy of calculations.

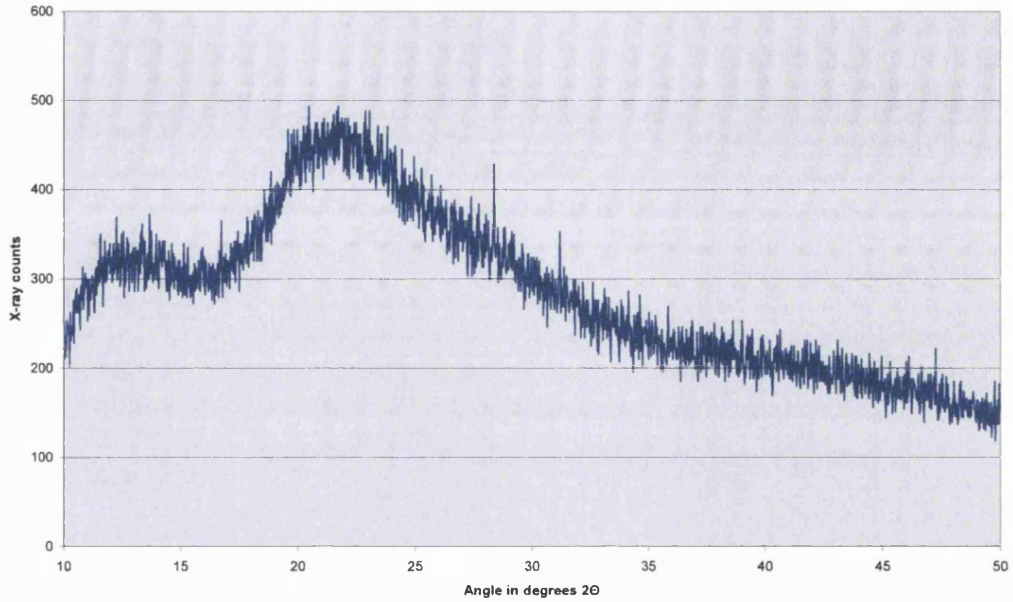

Figure 3.9 A sample XRPD scan of an indomethacin and PVP dispersion confirming that the dispersion is amorphous.

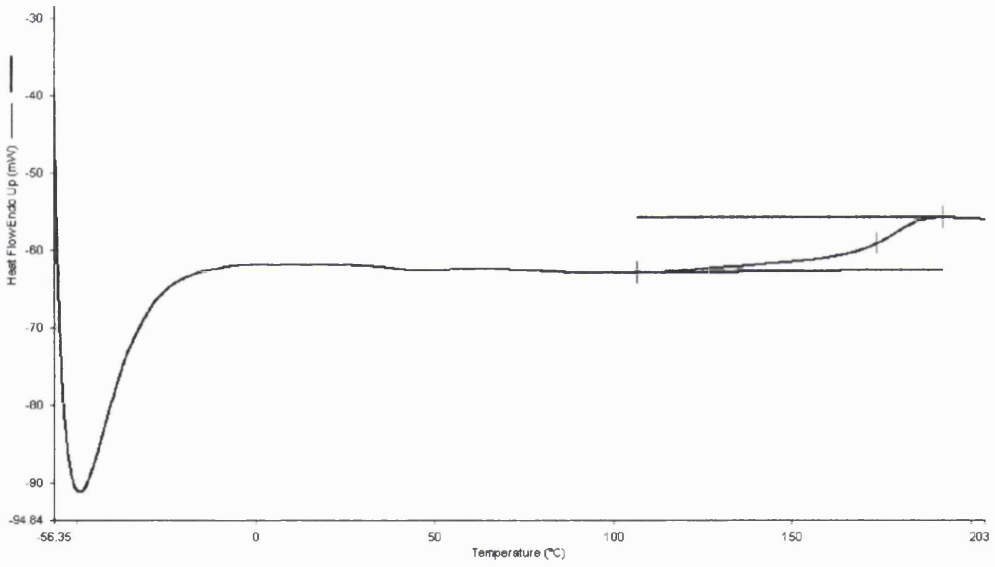

Figure 3.10 A sample DSC trace of an indomethacin, PVP and PAA dispersion showing a T_g with an onset of around 140 °C.

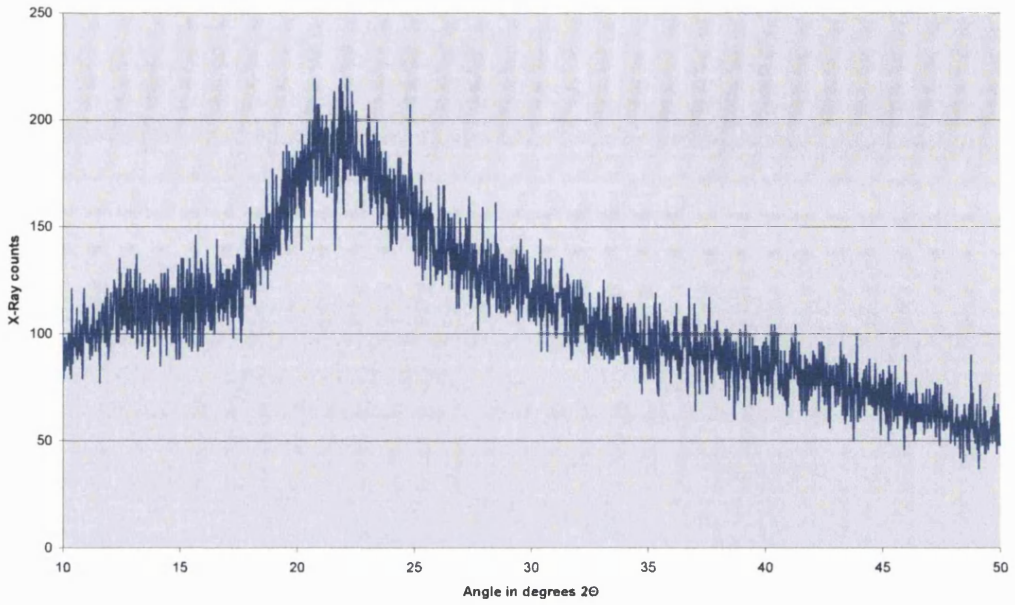

Figure 3.11 A sample XRPD scan of an indomethacin, PVP and PAA dispersion.

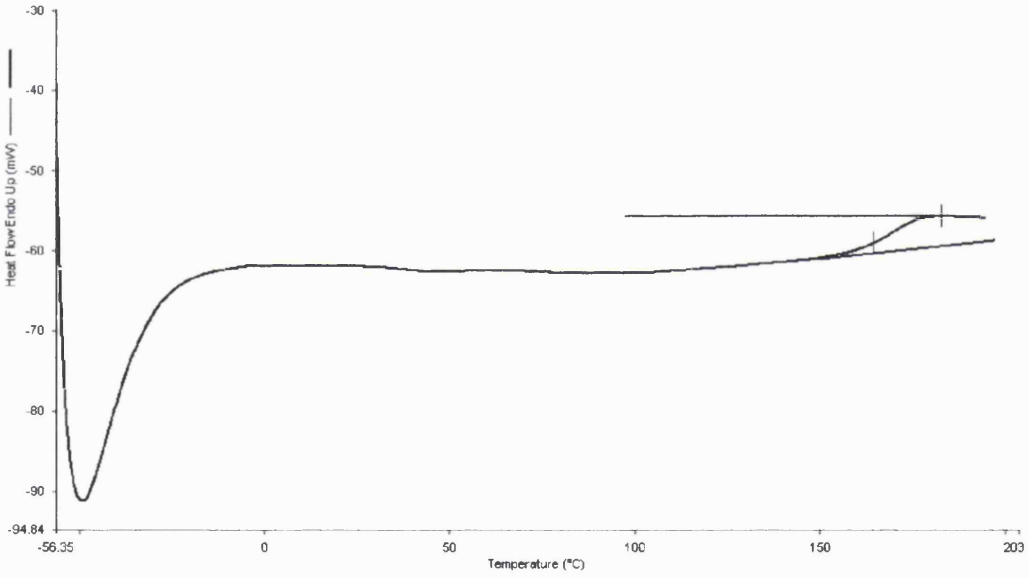

Figure 3.12 A sample DSC trace of an indomethacin, PVP and Eudragit S dispersion showing a T_g with an onset of around 170 °C.

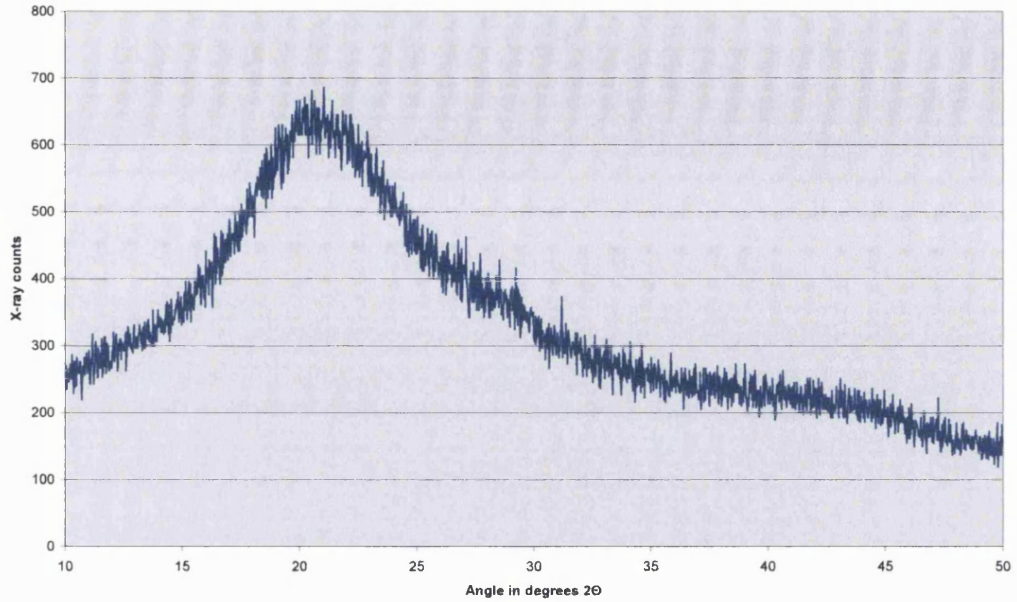

Figure 3.13 A sample XRPD scan of an indomethacin, PVP and Eudragit S dispersion.

Dispersion content	Percentage residual solvent	T_g (°C)
Indomethacin and PVP	2.1 ± 0.2	151.0 ± 1.3
Indomethacin, PVP and PAA	2.1 ± 0.1	143.1 ± 2.2
Indomethacin, PVP and Eudragit S	2.3 ± 0.1	164.3 ± 0.7

Table 3.5 Residual solvent content and the T_g for dispersions of indomethacin from made as per "Order Two", along with the same results for the indomethacin and PVP dispersion for comparison.

Dissolution studies performed on indomethacin dispersions produced by Order Two at pH 6.5 (Figure 3.14) showed considerable differences from those in Order One (Figure 3.6a). The indomethacin dispersed in PVP-Eudragit S was found to dissolve at pH 6.5. The dissolution of indomethacin dispersed in PVP and Eudragit S was not found to be significantly different from the dissolution of indomethacin dispersed solely in PVP (Student's *t*-test; $p < 0.05$). This was despite the presence of large particles (> 1 mm in diameter) of undissolved dispersion in the dissolution media after 45 min. These particles were still to be found after 8 h of mixing in the dissolution media.

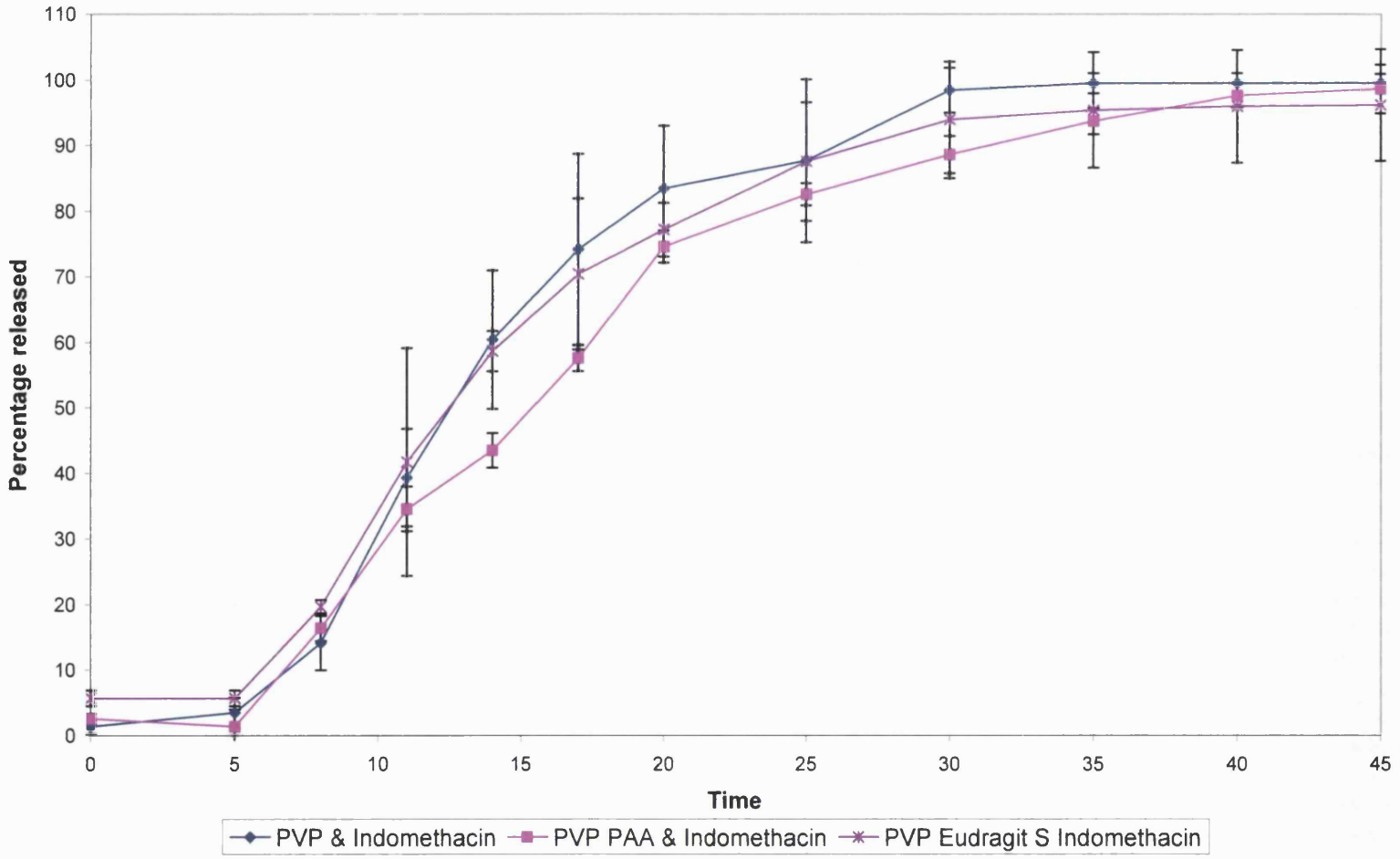

Figure 3.14 The dissolution of indomethacin from spray dried microparticles prepared using "Order Two" contained within hard gelatin capsules into pH 6.5 buffer over time expressed as percentage indomethacin released.

The most obvious reason for rapid dissolution of the indomethacin would be the existence of two separate domains, the first consisting of PVP and indomethacin and a second of Eudragit S and PVP. However there was insufficient evidence to support this hypothesis. The DSC results showed a single glass transition suggesting the presence of a single domain. It is possible that the separate domains could have been too small to be seen individually in the DSC. The domains could have had very similar T_g. One of the domains could have been dissolving in the other as the temperature increased. Poly(methyl methacrylate) and PEG grafted poly(methyl methacrylates) molecularly mixed together have been shown to display a single glass transition even though the system was known to be heterogeneous (Elmér and Jannasch, 2006). The effect of combining immiscible polymers on T_g has been investigated. It has been demonstrated that one component can have an effect on the other component's T_g despite there being minimal levels of interaction and the presence of separate domains (Thirtha *et al.*, 2006).

In a similar manner to that observed in the indomethacin, PVP and Eudragit S dispersion the release of indomethacin from the indomethacin, PVP and PAA dispersion produced by the Order Two method was also remarkably similar to the release form the indomethacin and PVP dispersion. The only time point that was statistically significantly different from the indomethacin and PVP dispersion was that at 14 min.

3.3.6 Order Three Results and Discussion

Batch three consisted solely of an indomethacin, PVP and Eudragit S dispersion, which was compared with the indomethacin and PVP dispersion created for Batch One. Again the dispersion produced was shown to be amorphous by both DSC and XRPD. Dissolution studies in pH 6.5 buffer demonstrated that indomethacin release was not significantly different from that of an indomethacin and PVP dispersion (Figure 3.15). From this it was decided that an examination of the dissolution in pH 7 buffer was unnecessary.

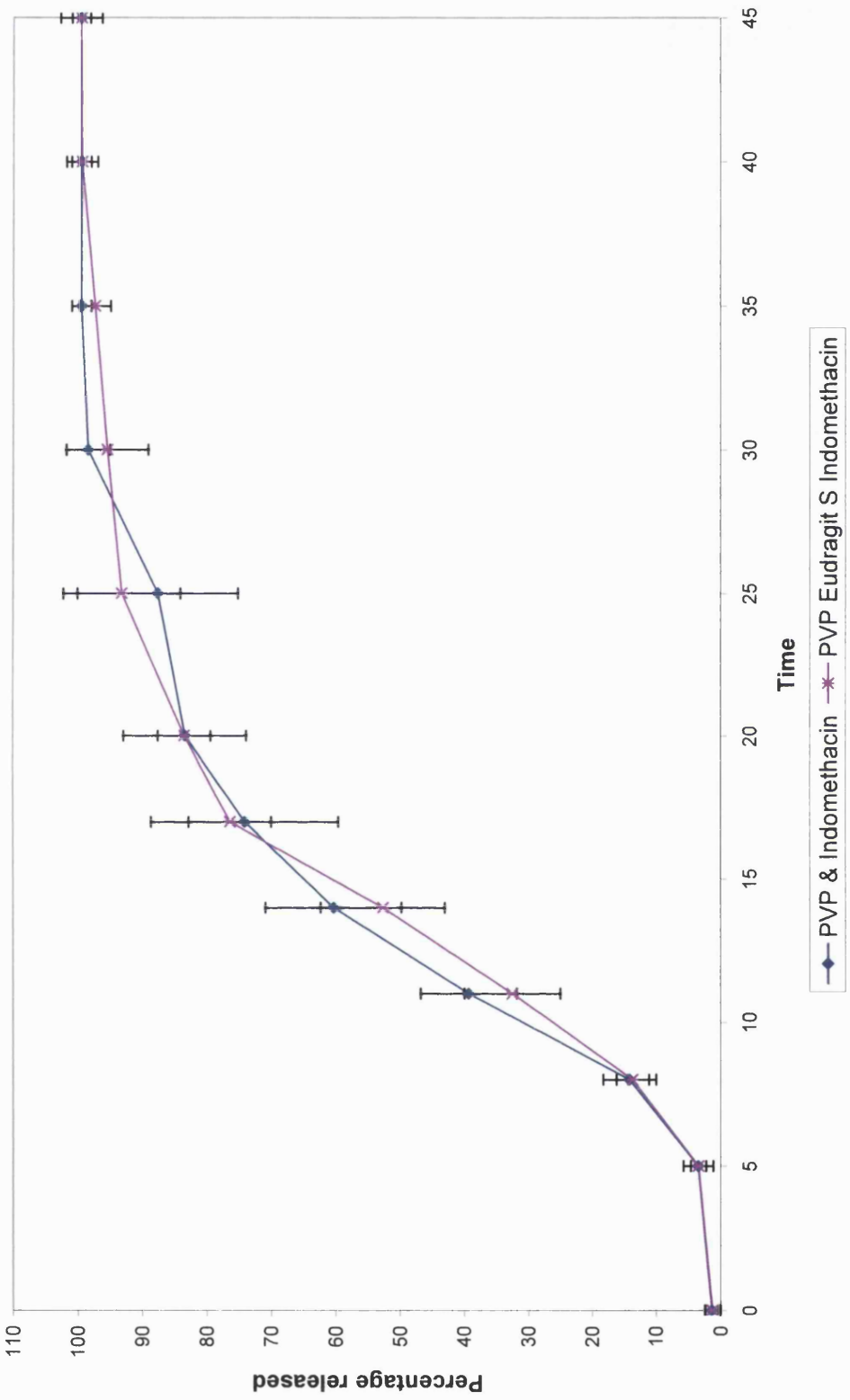

Figure 3.15 The dissolution of indomethacin from spray dried microparticles prepared using "Order Three" contained within hard gelatin capsules into pH 6.5 buffer over time expressed as percentage indomethacin released.

3.3.7 GENERAL DISCUSSION OF ALL DISPERSIONS

For convenience, the order in which the constituents of each dispersion were dissolved along with the solvents used to prepare each dispersion are provided in Table 3.6. As the indomethacin, PVP and Eudragit S dispersions showed the greatest difference in dissolution characteristics they will be the primary focus of this discussion. At pH 6.5 the release of indomethacin was unimpeded in the Order Two and Order Three indomethacin, PVP and Eudragit S dispersions. This was despite the presence of material that in the dissolution media that did not dissolve at pH 6.5. The indomethacin dissolution rate from these dispersions was shown to be very similar to that of the indomethacin and PVP dispersion. By comparison, the indomethacin in the indomethacin, PVP and Eudragit S dispersion produced by Order One did not show any dissolution at pH 6.5 over 5 h. A likely hypothesis is that differences in the interactions occurring in the dispersions lead to differences in dissolution and that these interactions were most likely occurring in the spray dryer feed solution as the order in which the components were dissolved was the only difference between the dispersions. The presence of undissolved material in the dissolution media suggests that the Order Two and Order Three indomethacin, PVP and Eudragit S dispersions were not homogenous. Areas rich in Eudragit S with some PVP probably give rise to the insoluble material. The indomethacin was probably in a PVP rich environment. This would then give rise to an indomethacin dissolution rate similar to that seen with the indomethacin and PVP dispersion.

Stage number	Order One	Order Two	Order Three
1	Ethanol	Ethanol	Ethanol
2	2nd Polymer	Indomethacin	Indomethacin
3	Water	PVP	Water
4	PVP	Water	2nd Polymer
5	Indomethacin	2nd Polymer	PVP

Table 3.6 The order in which the dispersion components were dissolved in the spray dryer feed solution.

Given that the indomethacin, PVP and Eudragit S dispersion produced by Order One showed pH dependent release it was expected that indomethacin, PVP and Eudragit S dispersion produced by Order Three would show pH-dependent release as indomethacin and Eudragit S were dissolved together prior to the addition of PVP. However, as

Figure 3.15 showed this turned out not to be the case, and again indomethacin release was not significantly retarded.

In terms of pH-dependent dissolution the indomethacin, PVP and Eudragit S system is not without merit. It produced smooth, spherical, relatively homogenous particles with good flowability.

3.4 CONCLUSIONS

The order of addition of constituents into the spray drying feed solution can have a significant effect on the dissolution profile of the resultant spray dried dispersion. At present this effect on dissolution is not predictable from the order of dissolution and no obvious mechanism presents itself. It is most likely that spectroscopic experiments such as FTIR may provide some answers as to the mechanisms by which these variations occur. As the particles that were produced using this method had good morphology there may be merit in developing the system further.

Chapter 4

The Use of a Second Excipient to Improve the Amorphous Stability of Griseofulvin and PVP Dispersions

4.1 INTRODUCTION

An ideal solid dispersion could also be considered to be a solid solution; a dispersion in which the components are molecularly dispersed within one another (Levy, 1963; Kanig, 1964; Chiou and Riegelman, 1971; Ford, 1986). When the carrier for a solid dispersion is an amorphous polymer this should result in the drug being held in the amorphous form which has been described as a "glass solution" (Chiou and Riegelman, 1971). As a consequence of this amorphous nature one would expect there to be an increase in apparent saturation solubility and dissolution rate (Hancock and Parks, 2000; Shekunov and York, 2000).

Polyvinylpyrrolidone (PVP) has a history of use as an agent in solid dispersions. It is highly water soluble across a wide range of pH and the only limiting factor to dissolution is the viscosity of the solution which is produced. This high solubility makes PVP an ideal candidate for the production of solid dispersions. PVP is also freely soluble in chloroform, ethanol, methanol and ketones. Moreover when given orally it has an excellent safety profile and has been used pharmaceutically for around 60 years (Rowe *et al.*, 2003). It is most commonly used as a binder in tablets.

PVP has a high glass transition temperature (T_g), and this may help stabilise drugs in the amorphous form (Hancock *et al.*, 1995; Van den Mooter *et al.*, 2000). Conversely, of the drugs that have been stabilised in the amorphous form by PVP many have been shown to interact with PVP through hydrogen bonding, with PVP being a proton acceptor and the drug acting as a proton donor. Examples of drugs shown to interact with PVP include indomethacin (Yoshioka *et al.*, 1995), frusemide (Akbuga *et al.*, 1988; Doherty and York 1989), ibuprofen (Najib *et al.*, 1986; Najib *et al.*, 1988) and piroxicam (Tantishaiyakul *et al.*, 1999).

4.1.1 GRISEOFULVIN

Griseofulvin is an antifungal drug active against dermatophyte fungi which acts by binding to newly formed keratin, it is generally prescribed to treat infections of the skin, scalp, hair and nails where topical therapy has failed or is inappropriate (Mehta, 2006). It appears to inhibit nucleic acid synthesis and is believed to have anti-mitotic and chitin synthesis activities. The melting point of griseofulvin is quoted in the British Pharmacopoeia (2005c) as being "about 220 °C". The British Pharmacopoeia (2005c) also states that griseofulvin is practically insoluble in water, with the definition of

practically insoluble being a requirement of in excess of 10 000 ml of solvent to dissolve 1 g of solute. Griseofulvin is also stated as being slightly soluble in ethanol and methanol; in effect the saturation solubility of griseofulvin is 1 g in approximately 100-1000 ml of these solvents.

Griseofulvin was chosen as the model drug for a variety of reasons; it is a well studied candidate for systems which increase dissolution rate and apparent saturation solubility, such as micronisation (Atkinson *et al.*, 1962; Kabasakalian *et al.*, 1970). Indeed the British Pharmacopoeia (2005c) gives a specification for griseofulvin particle size of "generally up to 5 μm in maximum dimension although larger particles which may occasionally exceed 30 μm may be present". Griseofulvin has been dispersed in PEG, in order to produce an ultra-micronised form and is commercially available in America from Pedinol Pharmacal Inc. as Gris-PEG®. By preparing the drug in this way the dosage has been reduced from 500 mg daily to 375 mg daily (Pedinol Pharmacal Inc., 2005). Sjökvist and Nyström (1988) demonstrated that the rate of griseofulvin release from solid dispersions of griseofulvin in PEG was directly dependent on the size of the drug particles contained within the dispersion. It is therefore reasonable to hypothesise that further improvements might be seen by reducing particle size still further and taking advantage of the amorphous form.

The structure of griseofulvin is shown in Figure 4.1. As can be seen it lacks a group capable of ionisation so it is not possible to manufacture different salts of the compound hence the use of micronisation to improve dissolution rate.

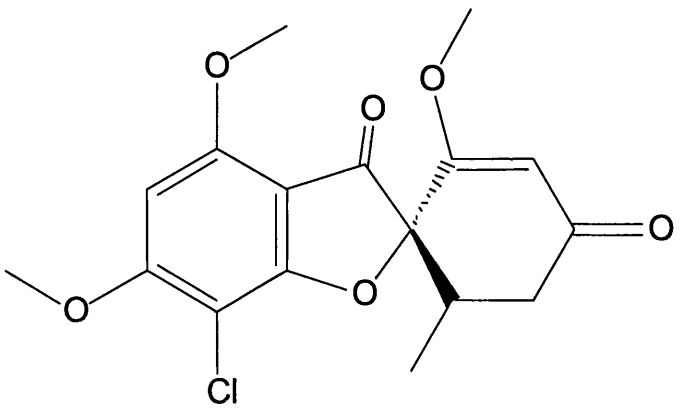

Figure 4.1 The structure of griseofulvin.

4.1.2 Choice of Second Excipients for the Preparation of Spray Dried Dispersions

Both griseofulvin (Figure 4.1) and PVP (Figure 4.2) can only act as proton acceptors for hydrogen bonding so there is limited scope for interactions between the two nor are they particularly miscible. This leads to phase separation and consequent recrystallisation despite their structural similarities (Shefter and Cheng, 1980; Nair *et al.*, 2001). Indeed it has been shown, under the high stress conditions of 40 °C and 69% RH that even when griseofulvin only makes up 30% by weight of a dispersion with PVP after 90 days storage no amorphous material remains (Vasanthavada *et al.*, 2005). Logically this recrystallisation will result in a decrease in the dissolution rate of the drug from the dispersion.

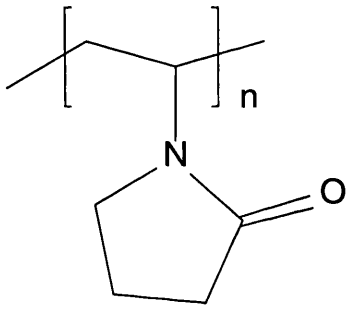

Figure 4.2 The structure of the basic repeat unit of polyvinylpyrrolidone (PVP).

It was decided that in order to stabilise griseofulvin in the amorphous form specific interactions, i.e. hydrogen bonding between griseofulvin and the excipient(s) forming the dispersion were required. As the nature of this thesis was to investigate the effect of the use of small amounts of functionalised excipients in solid dosage forms the decision was made to investigate the use of a minor secondary excipient which could hydrogen bond with both PVP and griseofulvin, and thus act as a bridge between the two to stabilise griseofulvin in the amorphous form.

The body of work presented in Chapter 3 demonstrated that relatively small amounts of secondary polymeric excipients can have an effect on dissolution by conferring their pH-dependent release on the dispersion mass. Since solid dispersions are designed to induce very rapid dissolution the decision was made to examine the effect that poly(2-hydroxypropylmethacrylate) (PHPMA), sucrose and poly(acrylic acid) (PAA) might have on the amorphous stability of griseofulvin dispersions. PHPMA and sucrose were chosen, as their dissolution properties are less subject to change across different levels of pH when compared with the Eudragit acrylate polymers studied in Chapter 3 and

they have hydroxyl groups which could act as proton donors in hydrogen bonding. PHPMA has shown to have a specific interaction with PVP. It is also miscible with PVP and blends of the two polymers show an elevated T_g (Kuo *et al.*, 2004). PAA has a lower pK_a value than the Eudragit polymers and thus is more likely to be soluble at a lower pH than Eudragit L or Eudragit S. Furthermore, PAA has a higher ratio of carboxylic acid groups to molecular mass than either of the Eudragits therefore it should act as a more effective stabilising agent.

In systems containing the same pair of excipients it was hypothesised that the relative concentration of the PVP to the second excipient would alter the stability of the dispersion. PVP was not expected to interact with griseofulvin as the two tend to phase separate (Vasanthavada *et al.*, 2005). One would therefore expect that increasing the concentration of the second excipient whilst decreasing that of PVP could reduce the amount of phase separation and, consequently, decrease the rate of recrystallisation. Conversely, as PVP has a higher T_g than the secondary excipients decreasing the concentration of PVP might increase the rate or level of crystallisation.

4.1.3 Preparation of Solutions

As in Chapter 3 one of the primary concerns for the preparation of spray dried solid dispersions that consist of a single phase is the preparation of a single phase solution. The dispersions will consist of a drug with poor water solubility combined with two relatively water soluble excipients. The first set of experiments performed consisted of small-scale investigations in to the solubility of the different components, both on their own and in combination. Order of addition and the consequent interactions in solution prior to spray drying may also affect stability. Changes in the order of dissolution of PVP, Eudragit S and indomethacin prior to spray drying appeared to result in differences in interactions in the solid state, which then led to changes in the pH at which dissolution occurred.

4.1.4 The Amorphous Stability of Spray Dried Griseofulvin

Griseofulvin has been used on a number of occasions as a model drug for the examination of systems purporting to increase the apparent saturation solubility and/or dissolution rate of poorly water soluble drugs (Chiou and Riegelman, 1969b; Kaur *et al.*, 1980a; Kaur *et al.*, 1980b; Bodmeier and Paeratakul, 1989). In this chapter griseofulvin is to be used as the model drug for the production of solid dispersions. The

crystalline griseofulvin was characterised as supplied to provide data that would allow the quantitative assessment of the stability of amorphous griseofulvin. "Quench-cool" experiments were performed to characterise pure amorphous griseofulvin. Amorphous griseofulvin prepared by spray drying was then characterised and compared with the crystalline griseofulvin and the "quench-cool" griseofulvin. Analysis of the crystallisation of spray-dried amorphous griseofulvin on storage was then performed.

4.2 METHODS

4.2.1 SMALL SCALE SOLUBILITY EXPERIMENTS

As was discussed in Section 3.1.2 solvents for spray drying were chosen for their safety characteristics. Water and ethanol are both Class 3 solvents and the solubility of griseofulvin in both solvents was examined. Acetone is also a Class 3 solvent and was also considered for spray drying in the event that ethanol and/or water proved to be unable to dissolve all the components of a dispersion. Ethanol and water are miscible with one another as are acetone and water.

The target concentration for the spray dried feed solutions was 10%. Griseofulvin (100 mg) was accurately weighed into a 2 ml glass vial containing a magnetic flea and sealed with a rubber septum. Acetone (1 ml) was then added and the vial stirred at room temperature for 8 h. The appearance of the suspension/solution was then examined. In the event that a significant amount of the griseofulvin had dissolved the supernatant was filtered through a 0.45 μm filter. The solution of griseofulvin (100 μl) was then diluted with acetone (to 1ml) and the concentration of griseofulvin in solution was assessed by UV spectroscopy. A calibration curve consisting of 4 data points between 1-5 mg/ml was used. The experiment was repeated using ethanol as the solvent instead of acetone.

A bulk of griseofulvin solution (100 ml) was prepared and aliquots (400 μl) were measured into vials (2 ml) and magnetic fleas were added. Each of the excipient solutions (200 μl) in water prepared as per Section 3.2.2 was added to a separate aliquot. The experiment was then repeated with fresh griseofulvin aliquots and the PVP and secondary excipient solutions were added in a variety of orders along with extra solvent where required, in order to determine the concentrations of each it would be possible to dissolve together.

4.2.2 CHARACTERISATION OF GRISEOFULVIN AND OTHER DISPERSION COMPONENTS

Characterisation of crystalline griseofulvin was performed using differential scanning calorimetry (DSC) to find an onset temperature and enthalpy (ΔH) for the melt. DSC scans were performed on griseofulvin samples across the range 30-250 °C at a rate of 10 °C/min as described in Section 2.5. The peak associated with the melting of griseofulvin was located and the ΔH of the melt was calculated using the Pyris Thermal Analysis Software (version 3.81) for Windows. The average of four scans was used to calculate both the onset temperature of the melt (T_m) and the ΔH. The experiment was then repeated across the range 30-270 °C at a rate of 200 °C.

Characterisation of the crystalline material was also performed using X-ray powder diffraction (XRPD). As discussed in Section 2.7.1 particle size can have an unpredictable effect on the XRPD peak height and area with smaller particle sizes tending to produce broader peaks (Hurst *et al.*, 1997). After spray drying, the particle size was expected to be less than 10 μm. Therefore the griseofulvin was sieved through a 35 μm sieve to reduce the particle size to a value more akin to that of the spray dried samples. Reducing particle size also has the advantage of reducing the risk of preferred orientation as was discussed in Section 2.7.2 (Campbell-Roberts *et al.*, 2002). Ten XRPD scans were taken and used to generate controls for later XRPD experiments using the methodology described in Section 2.7.2. A sample scan of stock griseofulvin is shown in Figure 4.3.

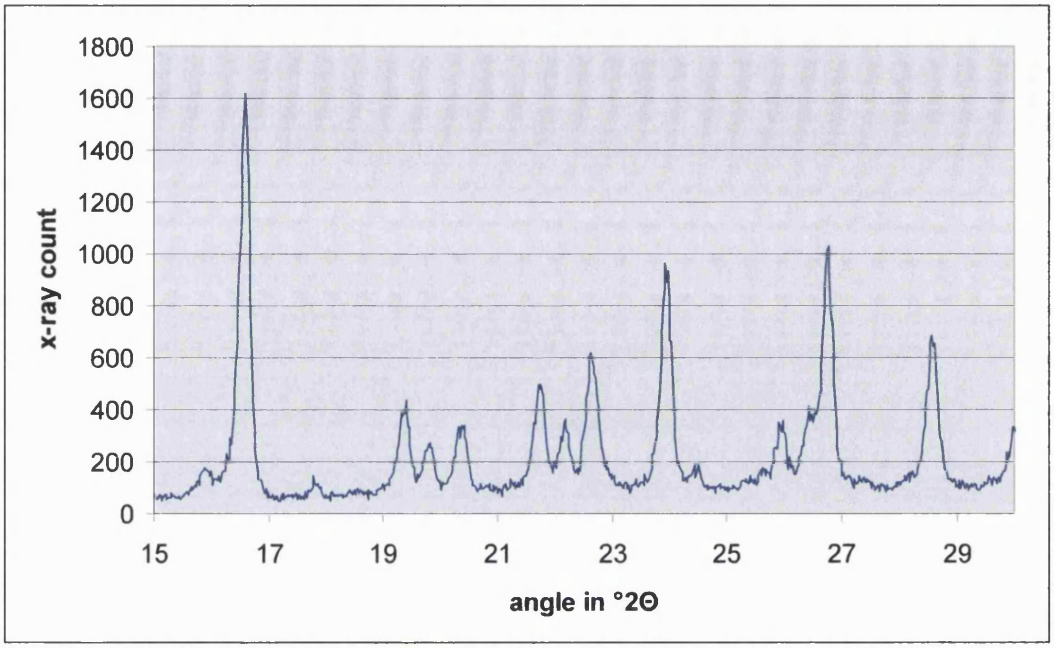

Figure 4.3 A sample XRPD scan of sieved crystalline griseofulvin.

In order to characterise the amorphous form of griseofulvin a sample was slowly melted in a crucible over a Bunsen burner. The molten griseofulvin was then poured into a flask filled with liquid nitrogen. Care was taken when heating to avoid degradation of the griseofulvin which was observed as a colour change. The excess liquid nitrogen was decanted and the amorphous solid was tested using DSC performed across the range 30-250 °C at a rate of 10 °C/min as in Section 2.5.3.

The excipients were characterised by DSC at both 10 °C/min and 200 °C/min. The experiments took the form of "heat-cool-heat" experiments. The sample was prepared as in Section 2.5.3. The sample was heated up to 100 °C and held at that temperature for 5 min in order to drive off any solvent. The sample was then cooled to -50 °C and reheated to 150 °C. If the expected transition (melt or T_g) was not observed the experiment was repeated with the second heating step taking the sample to 200 °C.

A sample of PVP (30 mg) was also analysed using Dynamic Vapour Sorption (DVS) and Near Infrared Spectroscopy (NIRS) as per Section 2.10. The RH cycle used was 0% RH for 300 min (5 h), 30% RH for 240 min (4 h) and finally 0% RH for 240 min (4 h). Whilst in the DVS-NIRS, NIR spectra of the sample were taken every 15 min as described in Section 2.10.

4.2.3 METHOD OF PRODUCTION AND TESTING OF SPRAY DRIED GRISEOFULVIN

Griseofulvin (6.38 g) was dissolved in stirred acetone (240 ml) in a 500 ml conical flask for a period of 2 h. The solution was then spray dried using the SD Niro as described in Section 2.3. An inlet temperature of 65 °C was used with the outlet set at 45 °C (both ±3 °C). The nitrogen flow rates were 20 kg/h and 2 kg/h for the drying and pulverising steps respectively. The feed rate was held at 20% (±2%) of the maximum feed rate. This is approximately 3 ml/min.

Residual solvent levels were determined by thermogravimetric analysis (TGA) as described in Section 2.4 which was run between 30-150 °C at a rate of 10 °C/min. DSC at 10 °C/min across the range 30-250 °C was performed on the sample to examine the effect that the presence of solvent would have on the T_g, and crystallisation.

XRPD using equipment and methods described in Section 2.7, was performed on the sample after spray drying and at regular intervals for seven days to observe the extent of crystallisation. In order to quantify crystallinity three peaks on the XRPD scans of the crystalline griseofulvin produced in Section 4.2.1 were selected; a peak found at around 16-17 °2Θ, a peak around 24 °2Θ and a peak just above 28 °2Θ and these peaks were chosen as they were the largest peaks available that also displayed reasonable baseline resolution (Figure 4.3). The peaks were integrated, using a Lorenzian distribution as described in Section 2.7, and summed to give a total peak area which could then be compared with the peak area of XRPD scans of spray dried griseofulvin.

4.2.4 PREPARATION OF GRISEOFULVIN DISPERSION SET ONE

Table 4.1 lists the contents of each sample prepared in Set One. Accurately weighed griseofulvin was dissolved in stirred acetone (240 ml) in a 500 ml conical flask. After 2 h distilled water (100 ml) was then added and solution allowed to mix for a further 15 min. This was followed by the addition of the secondary excipient as a solid. Once the secondary excipient had dissolved the PVP was added. Dispersion 4.1.3 and 4.1.4 (both containing PAA) required the addition of extra water in order to dissolve the PAA and PVP, and in total approximately 250 ml of water was used to create solutions containing all the constituents.

Dispersion number	Amount of griseofulvin (g)	Amount of PVP (g)	Details of secondary component
4.1.1	6.0	4.0	None
4.1.2	4.0	6.0	None
4.1.3	6.0	3.0	1.0 g of PAA
4.1.4	4.0	4.5	1.5 g of PAA
4.1.5	6.0	3.0	1.0 g of PHPMA
4.1.6	4.0	4.5	1.5 g of PHPMA
4.1.7	6.0	3.0	1.0 g of sucrose
4.1.8	4.0	4.5	1.5 g of sucrose

Table 4.1 The composition of each spray dried dispersion in Set One.

The mixtures were then spray-dried in the SD Niro system. The parameters used were the same as those in Chapter 3; an inlet temperature of 65 °C and an outlet of 45 °C were used, with the nitrogen atomisation rate being 2 kg/h and the drying rate being 20 kg/h. The feed rate was held at 20% (±2%) of the maximum feed rate which was approximately 3 ml/min. The samples were then split into five approximately equal amounts and transferred to 4 ml Waters vials for storage at the required conditions.

4.2.5 Storage Conditions and Analysis

Three desiccators were prepared containing approximately 200 g of phosphorous pentoxide (P_2O_5) were used to provide 0% RH conditions and were stored at room temperature (RT), 40 °C and 50 °C. Two further desiccators were prepared using 250 ml of a saturated solution (with excess solid) of magnesium chloride ($MgCl_2$). These desiccators were stored at room temperature and 50 °C. At room temperature $MgCl_2$ produces a RH of between 32.8% and 33.3%, whilst at 50 °C it produces 30.5% RH (Nyqvist, 1983).

Samples were tested for weight loss using thermogravimetric analysis (TGA) with the weight loss being ascribed to residual solvent. The samples were tested across the temperature range 30-150 °C at a rate of 10 °C/min. The dispersions were then separated into five equal portions and placed in 4 ml Waters vials. These were stored in the desiccators prepared as above. The samples were tested for crystallisation using

XRPD which has been described in detail in Section 2.7 and Section 4.2.3. Briefly, the peak found between 16-17 °2Θ, the peak around 24 °2Θ and the peak just above 28 °2Θ formed the basis of the analysis and the peak areas were compared with those found for pure griseofulvin to provide a percentage crystallisation. Samples of the dispersions were also examined by scanning electron microscopy (SEM).

4.2.6 PREPARATION OF GRISEOFULVIN DISPERSION SET TWO

Three dispersions were prepared as outlined in Table 4.2. Two of these dispersions (Dispersions 4.1.1a and 4.1.5a) were essentially the same as two of the dispersions produced as part of Set One (Dispersion 4.1.1 and 4.1.5), whilst the third is a slight variation. The feed solution was prepared as before, using the same amounts of acetone and storage took place at 50 °C, 0% RH. XRPD was again used to study crystallisation.

Dispersion number	Amount of Griseofulvin (g)	Amount of PVP (g)	Amount of PHPMA (g)
4.1.1a	6.0	4.0	0
4.1.5a	6.0	4.0	1.0
4.2.5	6.0	2.0	2.0

Table 4.2 The composition of each spray dried dispersion in Set Two.

4.2.7 PREPARATION OF GRISEOFULVIN DISPERSION SET THREE

Batch three consisted of 4 dispersions. The first, Dispersion 4.1.1b, contained griseofulvin and PVP and was prepared as per Section 4.2.1. Griseofulvin (6 g) was dissolved in stirred acetone (240 ml) before water (100 ml) was added followed by PVP (4 g). Three dispersions containing PHPMA were then prepared. Each contained the same constituents however the order in which these constituents were dissolved varied for each dispersion.

Dispersion 4.1.5b:

Griseofulvin (6 g) was dissolved in stirred acetone (240 ml). After 2 h of stirring, water (100 ml) was slowly added. PVP (3 g) was then dissolved over 15 min. Finally PHPMA (1 g) was added to the feed solution. The feed solution was then allowed to mix for a further 2 h before being spray dried as described previously.

Dispersion 4.3.5a:

PHPMA (1 g) was dissolved in distilled water (100 ml) in a 500 ml conical flask. After 2 h of stirring, PVP (3 g) was then added and allowed to dissolve for 15 min. Griseofulvin (6 g) was dissolved in stirred acetone (240 ml) in a separate 500 ml conical flask. After 2 h, the resultant solution was then slowly added to the stirred PVP and PHPMA solution. The resultant feed solution was then allowed to mix for a further 2 h before being spray dried.

Dispersion 4.3.5b:

Griseofulvin (6 g) was dissolved in stirred acetone (240 ml) in a 500 ml conical flask. After 2 h of stirring, distilled water (100 ml) was added and allowed to mix for 15 min. This was followed by the addition of PHPMA (1 g). After 2 h of stirring, PVP (3 g) was added and allowed to dissolve over 15 min. The resultant solution was then spray dried.

4.3 Results and Discussion

4.3.1 Results from Small Scale Solubility Tests

The saturation solubility of griseofulvin in acetone was found to be 36 mg/ml. Griseofulvin proved to be practically insoluble in water as expected and the solubility of griseofulvin in ethanol was insufficient to be useful in the preparation of spray dried griseofulvin. The further investigation of the solubility by UV spectroscopy of griseofulvinin in ethanol was not pursued.

Some of the results presented in here have already been presented in Chapter 3 and are reproduced for convenience. PAA M_w 2 000 was found to be insoluble in acetone, whilst it was found to be soluble in both ethanol and water to at least 100 mg/ml. PHPMA M_w 20 000 was found to be very poorly soluble in ethanol and acetone, however in water it was found to be soluble to 100 mg/ml though this took approximately 2 h. PVP was found to be highly soluble in both ethanol and water, to at least 100 mg/ml though it was found to relatively insoluble in acetone. Sucrose proved to be insoluble in acetone and very poorly soluble in ethanol, however it did prove to be soluble in water to at least 100 mg/ml.

A number of combinations of solvent systems were tried. Initially the combination of ethanol, which would dissolve all the polymers, with acetone, which would dissolve

griseofulvin, was considered. However it did not prove to be possible to produce a solution of griseofulvin and PVP in acetone and ethanol, which would allow the addition of further polymers. Whilst these small scale studies involved the combination of solutions, the intention was to add solid material to solutions as this would minimise the chance of precipitation when the solution was spray dried.

The use of acetone and water in combination proved to be more successful. It was possible to produce solutions of PHPMA and PVP in the solvent combination of water and acetone, however when a griseofulvin solution in acetone was used in place of pure acetone, one or more of the components fell out of solution. Increasing the amount of acetone relative to the amount of water tended to increase the risk of precipitation. Conversely the addition of more distilled water tended to reduce the amount of precipitation. When scaled up, the final solvent combination for spray drying proved to be 240 ml of acetone and 100 ml of water. The only dispersions that could not be prepared using this solvent system were those that contained PAA. These required an additional 100 ml of acetone to dissolve fully prior to spray drying.

4.3.2 THE CHARACTERISATION OF GRISEOFULVIN

When griseofulvin was tested by DSC at 10 °C/min the melt onset was 218.6 °C ± 0.3 °C and the ΔH was found to be 109.0 J/g ± 1.0 J/g. When the experiments were repeated at a scan rate of 200 °C/min the melt onset was 218.7 °C ± 0.4 °C and the ΔH was 108.7 J/g ± 0.7 J/g.

A representative DSC trace of the quench-cooled griseofulvin is shown in Figure 4.4. This DSC trace was produced within 30 min of the quenching. In Figure 4.4 three events can be seen, the first is a T_g with an onset temperature of 96 °C. This is followed by a recrystallisation event, and then the melt of the crystalline griseofulvin. Given that the T_g was more than 50 °C above room temperature it was expected that amorphous griseofulvin would be reasonably stable at room temperature after spray drying provided that the residual solvent in the sample was minimal.

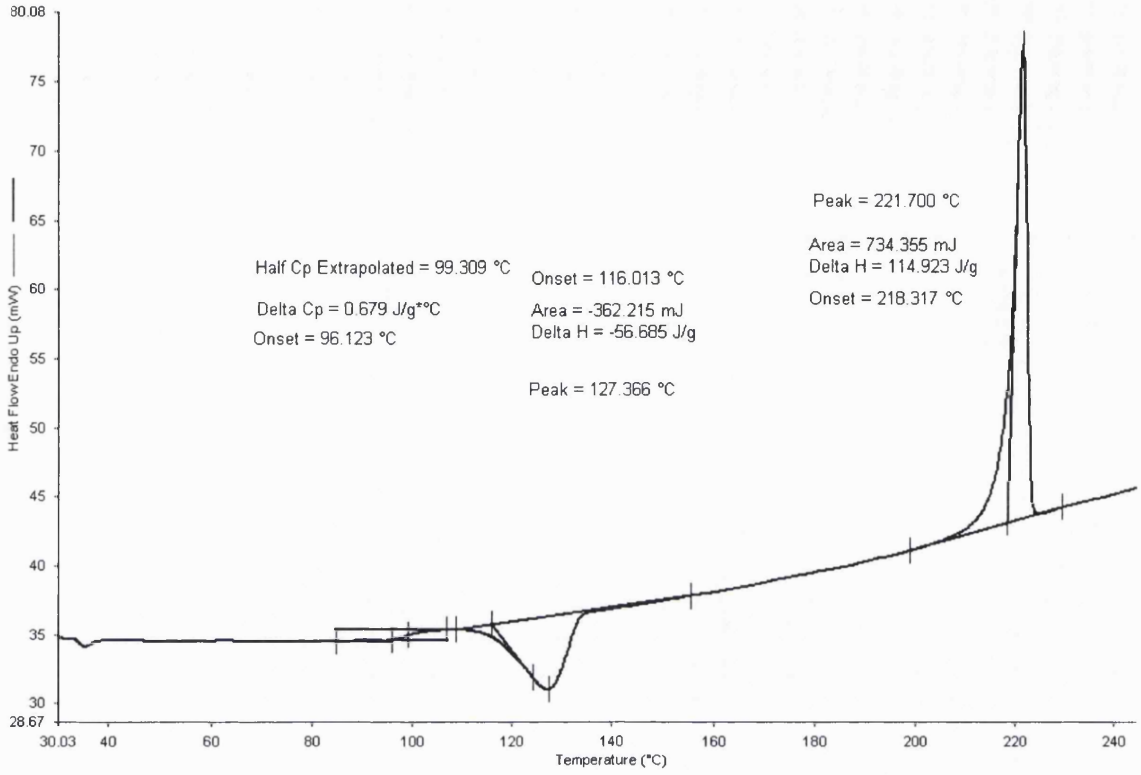

Figure 4.4 A DSC trace of quench-cooled griseofulvin run at 10 °C/min

4.3.3 THE STABILITY OF SPRAY DRIED GRISEOFULVIN

The weight loss of the spray dried griseofulvin observed in the TGA was 2.9% (from a single run). There was no evidence of degradation in the sample (charring or other colour change) and so this value was ascribed to the presence of residual acetone in the spray dried griseofulvin. This level of residual solvent was similar to the levels of residual solvent observed in dispersions produced in the previous chapter. DSC showed that on heating the spray dried amorphous griseofulvin tended to crystallise in a similar fashion to the griseofulvin produced by quench cooling (Figure 4.5). Unfortunately the solvent loss occured over the same temperature range (approximately 70-100 °C) as the recrystallisation. Quantification of the amorphous content by subtraction of the crystallisation enthalpy from the melt enthalpy proved to be unfeasible. The final melt of the griseofulvin had an onset of 216.8 °C and ΔH of 108.3 J/g, which compared favourably with the ΔH of the supplied griseofulvin calculated in Section 4.3.2 (Figure 4.5). The slight lowering of the melt onset temperature (T_m) was probably due to a small amount of amorphous griseofulvin or griseofulvin degradants. Impurities can lower the onset temperature of a melt. Impurities can also have an effect on the calculated lattice enthalpy. The T_g was not located initially, possibly due to the level of

residual solvent, which may also have had an effect on the calculated ΔH for the crystallisation. Repeat DSC runs were performed at 200-500 °C/min using the Pyris 1 DSC; however these showed no advantages in terms of inhibiting crystallisation, and merely created a broader and more obvious peak due to loss of solvent.

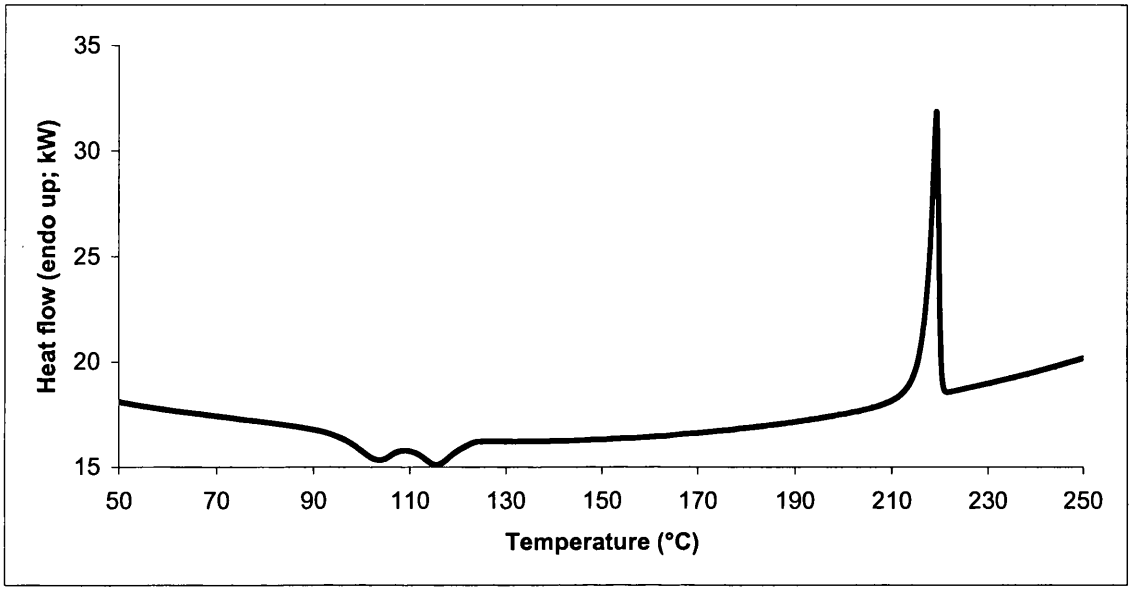

Figure 4.5 A DSC trace of freshly spray dried griseofulvin run at 10 °C/min.

XRPD indicated that the spray-dried sample was completely amorphous initially. This can be seen in Figure 4.6 (Day 0). The spray dried sample was then stored at 0% RH in a temperature controlled room (20 °C ± 3 °C) with XRPD analysis being performed every 1-2 days. The raw data from XRPD analysis showed a gradual crystallisation of the spray dried griseofulvin sample and this can also be seen in Figure 4.6. The T_g of spray dried griseofulvin was found to be 80.3 °C ± 2.4 °C by DSC at 10 °C/min, which was slightly lower than that for the quench-cooled griseofulvin presumably due to the presence of acetone. This was still more than 50 °C above room temperature and should mean that the griseofulvin is stable in the amorphous form (Hancock *et al.*, 1995).

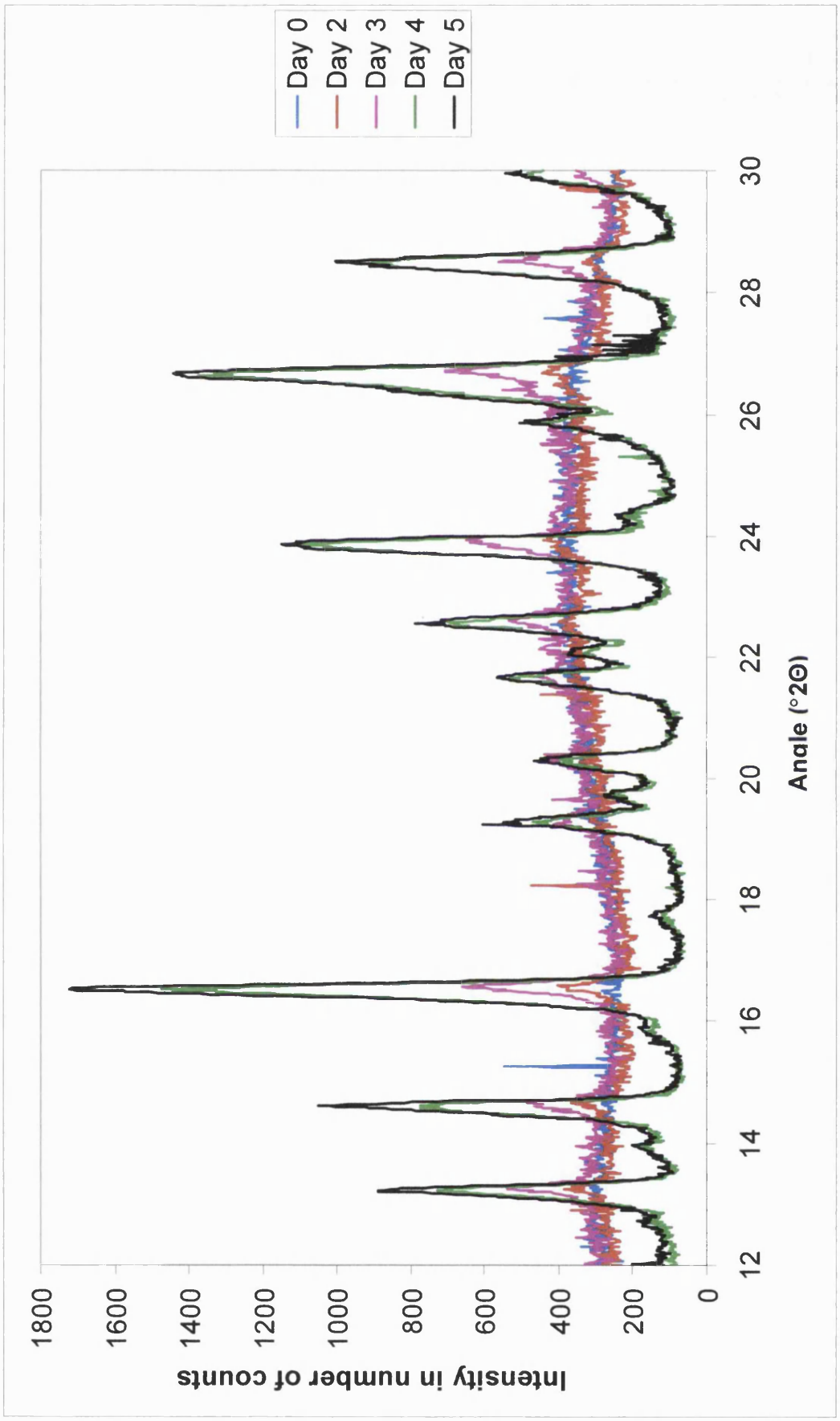

Figure 4.6 Spray dried griseofulvin XRPD raw data taken over 5 days.

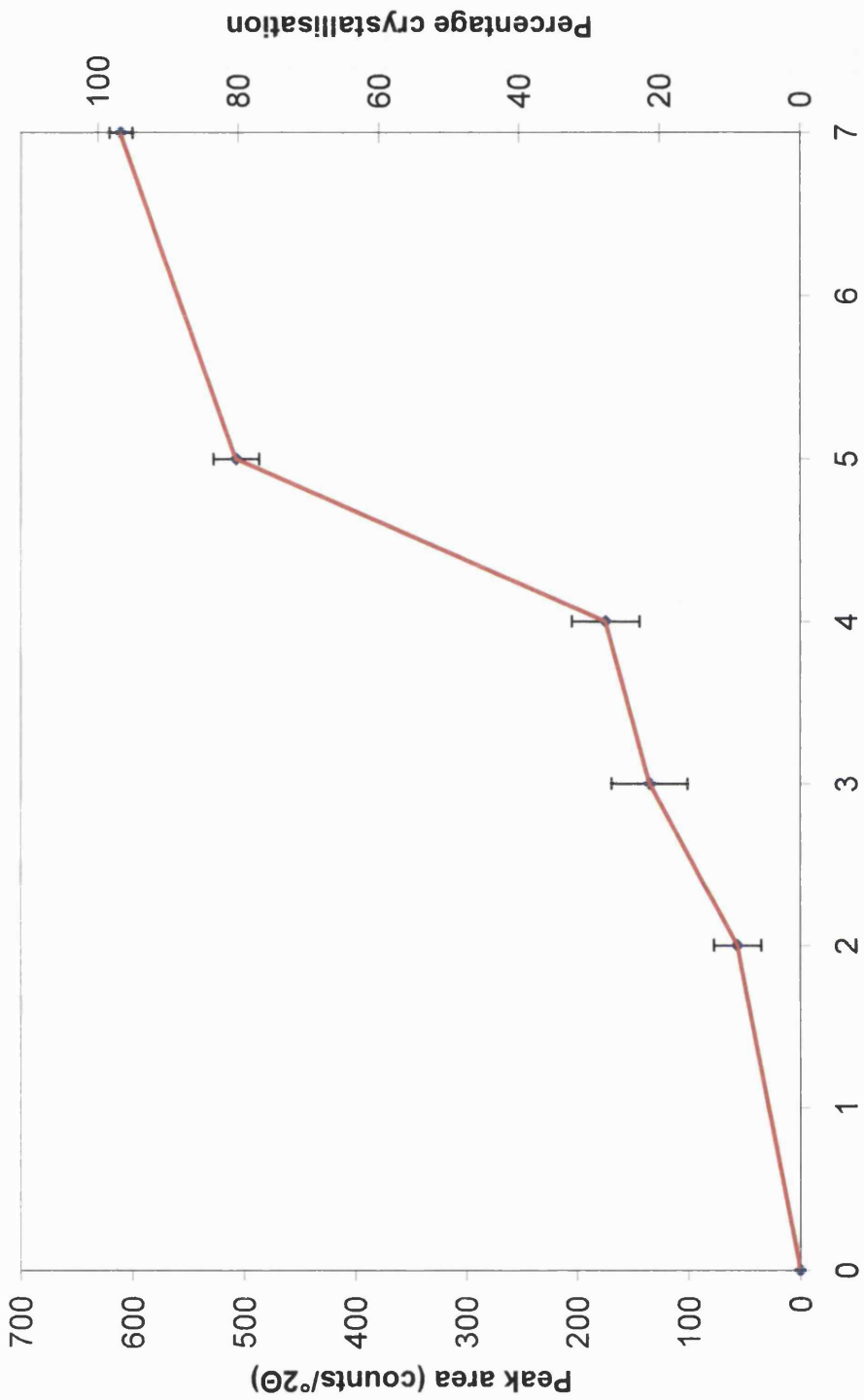

Figure 4.7 The crystallisation of spray dried griseofulvin over time.

Figure 4.7 shows the total peak area of the 3 peaks selected for analysis (the peak found between 16-17 °2Θ, the peak around 24 °2Θ and the peak just above 28 °2Θ) calculated using the methods described in Sections 2.7, 4.2.2 and 4.2.3. These values have also been expressed as percentage crystallisation, based on comparison with the supplied griseofulvin. There was some initial crystallisation within the first few days. This was most probably a mixture of nucleation and crystal growth. At some point on day 4 this reached a critical concentration and crystallisation proceeded very rapidly and near complete crystallisation occurred after 7 days. Inhibition of the initial crystallisation would appear to be one way in which one could stabilise the amorphous form.

The crystallisation process occurs despite griseofulvin having a T_g which, at approximately 80 °C, is more than 50 °C above room temperature. Generally this would be considered high enough to sufficiently reduce molecular mobility to confer stability of the amorphous form (Hancock *et al.*, 1995). The most likely rationale for crystallisation still occurring would be a localised reduction of T_g in the raw sample due to residual solvent. Owing to the initial DSC experiments at time zero, very few further DSC experiments were performed until day 7 when it was confirmed that the griseofulvin had fully recrystallised, as no recrystallisation could be detected on heating.

Number of Days of storage	Percentage residual solvent
0	2.9 ± 0.5
2	1.7 ± 0.3
3	1.4 ± 0.4
4	1.4 ± 0.4
5	1.4 ± 0.3
6	1.4 ± 0.4
7	1.2 ± 0.3

Table 4.3 The level of residual solvent found in spray dried griseofulvin.

4.3.4 Analysis of Dispersions Following Spray Drying

The yields from spray drying and the residual solvent present in each of the dispersions are shown in Table 4.4. In general the yield from each spray drying run was about 40-50%. These moderate yields were ascribed to the nature of the SD Niro spray dryer. It is a scale-up plant used to develop the parameters for industrial manufacturing. It is

described by Niro as being used to spray dry 100-200 g of solid material rather than the 10g of solid material that is being spray dried here. However the advantage of being able to use organic solvents with this spray dryer outweighs this disadvantage. On examination of the spray dryer after each run large deposits of solid material were found in the pipe between the drying chamber and the cyclone whilst very little was deposited between the cyclone and the bag filters. The initial XRPD results showed all the dispersions to be amorphous after storage for 24 h under vacuum at room temperature.

Dispersion number (details of second excipient, where applicable)	**Percentage Yield**	**Percentage residual solvent**
4.1.1	40.5	1.9 ± 0.2
4.1.2	34.6	2.2 ± 0.3
4.1.3 (PAA)	50.2	2.3 ± 0.3
4.1.4 (PAA)	27.4	1.8 ± 0.1
4.1.5 (PHPMA)	44.4	1.5 ± 0.4
4.1.6 (PHPMA)	50.4	1.4 ± 0.3
4.1.7 (sucrose)	32.5	2.4 ± 0.5
4.1.8 (sucrose)	48.4	2.4 ± 0.3

Table 4.4 Yields and levels of residual solvent found in spray dried solid dispersions.

TGA results generally showed a similar level of solvent to be present in each dispersion. Hence stability test results could be compared between the different griseofulvin dispersions as the plasticising effect of residual solvent was expected to be similar. After storing the dispersions under vacuum (24 h, ambient temperature) only a minimal loss of residual solvent was observed (0.1-0.5%). Scanning electron microscope images of the dispersion particles are shown in Appendix One. Particles were generally found to be smaller than 10 μm and spherical with a smooth surface.

Dispersion number (details of second excipient, where applicable)	**T_g (°C)**
4.1.1	108.0 ± 1.5
4.1.2	121.4 ± 3.4
4.1.3 (PAA)	90.4 ± 2.0
4.1.4 (PAA)	112.6 ± 1.3
4.1.5 (PHPMA)	97.9 ± 1.4
4.1.6 (PHPMA)	104.3 ± 0.4
4.1.7 (sucrose)	90.2 ± 0.2
4.1.8 (sucrose)	108.0 ± 3.7

Table 4.5 The T_g of spray dried griseofulvin dispersions.

Table 4.5 shows the T_g for the various dispersions. The presence of a third constituent in the dispersion reduced the overall T_g of the dispersion. All the tertiary components have a considerably lower T_g than that of PVP. Despite the possibility of increased intermolecular non-covalent interactions resulting in a higher T_g (as was seen with the PVP and PAA precipitates in Section 3.3.3) the tertiary dispersions generally have a T_g that is at least 10 °C lower than that of Dispersion 4.1.1 and Dispersion 4.1.2 (the binary dispersions). This was also unexpected given that elevation of T_g has been observed in blends of PVP with PAA (Lau and Mi, 2002) and PVP with PHPMA (Kuo *et al.*, 2004).

DSC studies of amorphous griseofulvin presented in Section 4.3.1 demonstrated that amorphous griseofulvin has a propensity to crystallise on heating (Figures 4.4 and 4.5). Therefore an initial DSC study was performed on the first spray dried griseofulvin-PVP dispersion, listed as Dispersion 4.1.1. Figure 4.8 shows a sample DSC trace of Dispersion 4.1.1. The first major event was an endotherm that was most likely to relate to solvent loss as it started at a similar temperature to the temperature at which weight loss was seen in the TGA. This was immediately followed by an exotherm which was most probably due to the recrystallisation of griseofulvin from the dispersion. Finally there was a melt endotherm related to griseofulvin which was most likely due to the recrystallisation that occurred earlier in the heating stage. As the loss of solvent and the griseofulvin recrystallisation could not be separated it was not possible to accurately calculate the percentage of crystalline material that was present in the sample prior to

heating in the DSC. Hence the decision was made to continue to use XRPD to analyse the rate at which griseofulvin crystallises.

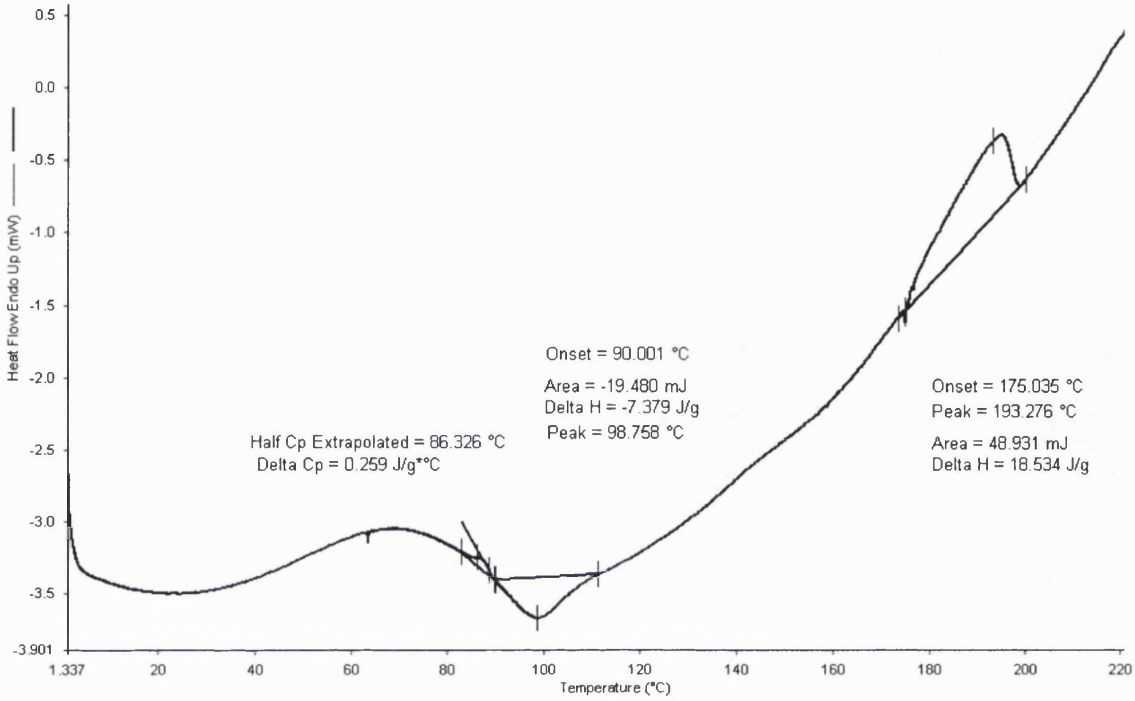

Figure 4.8 A DSC trace of a dispersion containing griseofulvin and PVP in a ratio of 60:40.

4.3.5 X-RAY POWDER DIFFRACTION RESULTS FOR DISPERSION SET ONE WHEN STORED AT 0% RH

Samples of each dispersion (600-800 mg) were stored at 50 °C, 0% RH and 30% RH, 40 °C, 0% RH and room temperature 0% RH and 30 % RH for 13 weeks, with an initial analysis to confirm that the dispersions were amorphous and further analyses were preformed after 3 weeks and 13 weeks.

After 3 weeks storage at 50 °C, 0% RH, crystalline griseofulvin was detected in all the samples (Figure 4.9). In comparison with Dispersion 4.1.1 and Dispersion 4.1.2 (griseofulvin and PVP binary dispersions), Dispersion 4.1.3 and Dispersion 4.1.4 which both contained PAA and Dispersion 4.1.7 and Dispersion 4.1.8 which both contained sucrose all proved to be more unstable. Sucrose was more detrimental to amorphous stability than PAA. The addition of PHPMA (in Dispersion 4.1.5 and Dispersion 4.1.6) was observed to have beneficial effect on stability. The T_g of each of the tertiary

dispersions were remarkably similar with only a 7 °C difference between Dispersion 4.1.3 (PAA), Dispersion 4.1.5 (PHPMA) and Dispersion 4.1.7 (sucrose) (Table 4.5). The difference in stability was therefore not linked to T_g.

It interesting to note that the percentage crystallisation after 3 weeks was essentially the same for each pair of samples. For example; the griseofulvin in Dispersion 4.1.1 was 33.1% crystalline and in 4.1.2 it was 33.6% crystalline. The significance of this observation is not clear. The rate of crystallisation could be related to some intrinsic property of the components of the dispersion or the way in which they are interacting and not only to their relative concentrations. If a large amount of griseofulvin in each sample was not being stabilised by the other constituents of the dispersion in some way one would expect there to be a far more rapid rate of crystallisation in the dispersions consisting of griseofulvin 60% w/w as this "free" griseofulvin should crystallise within the first 7 days as was seen with the spray dried griseofulvin samples in Section 4.3.3.

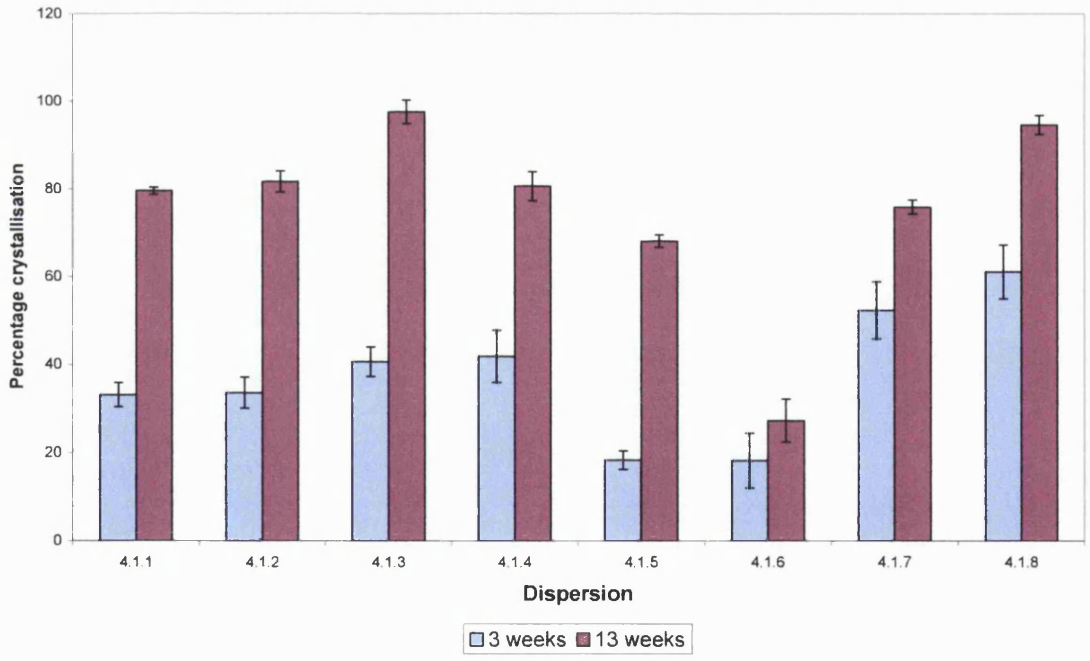

Figure 4.9 The percentage crystallisation of griseofulvin dispersions stored at 50 °C and 0% RH for 13 weeks.

After 13 weeks of storage at 50 °C, 0% RH, 79.6% of the griseofulvin in Dispersion 4.1.1 and 81.8% of the griseofulvin in Dispersion 4.1.2 had crystallised. Of the two dispersions containing PAA, Dispersion 4.1.3 displayed near complete griseofulvin crystallisation (97.6%), whilst the level of crystallisation of griseofulvin in dispersion

4.1.4 (80.8%) was not significantly different to that of Dispersion 4.1.2 (via a two tailed Student's *t*-test; $p > 0.05$). Of the sucrose dispersions, Dispersion 4.1.7 contained 75.9% crystalline griseofulvin whilst Dispersion 4.1.8 contained 94.6% crystalline material. It was surprising that Dispersion 4.1.8, which contained a greater concentration of PVP crystallised to a greater degree than Dispersion 4.1.7. The dispersion did not display any of the peaks associated with sucrose crystallisation and it was unlikely that peaks associated with sucrose crystallisation could be masked by griseofulvin peaks as they result in different diffraction angles. Moreover, should any sucrose crystallisation have occurred this could not be mistaken for griseofulvin crystallisation (Figure 4.10). The melting point of sucrose is around 180 °C (Yudkin *et al.*, 1973). Results of DSC scans of Dispersion 4.1.7 showed the absence of an endothermic peak corresponding to this melt therefore the sucrose in this dispersion was not crystalline. It is possible that this unexpected result was due to plasticisation of the dispersion by sucrose which results in increased levels of crystallisation.

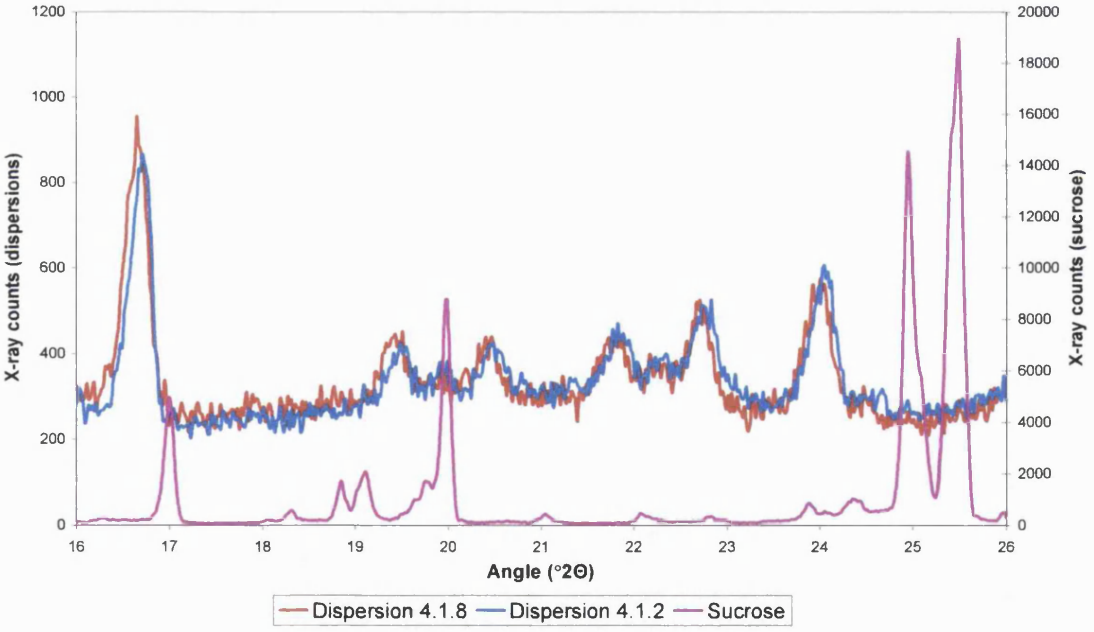

Figure 4.10 An example of the XRPD peaks seen within Dispersions 4.1.2 and 4.1.8 compared with those seen in a pure sucrose sample.

The only additive to display any significant beneficial effects at 50 °C in these samples was PHPMA. In Dispersion 4.1.5 and Dispersion 4.1.6, 68.2% and 27.3% of the griseofulvin had crystallised respectively. Overall these differences suggested that the use of sucrose or PAA in the dispersion tended to be detrimental since a significant

difference in crystallisation was seen. Conversely, the use of PHPMA in Dispersion 4.1.6 and to a lesser extent Dispersion 4.1.5 clearly resulted in a significant improvement in the stability of amorphous griseofulvin. Given that all three of these additives can hydrogen bond with either griseofulvin or PVP and that there was little difference in T_g it is not entirely clear as to why there are such large differences in amorphous stability.

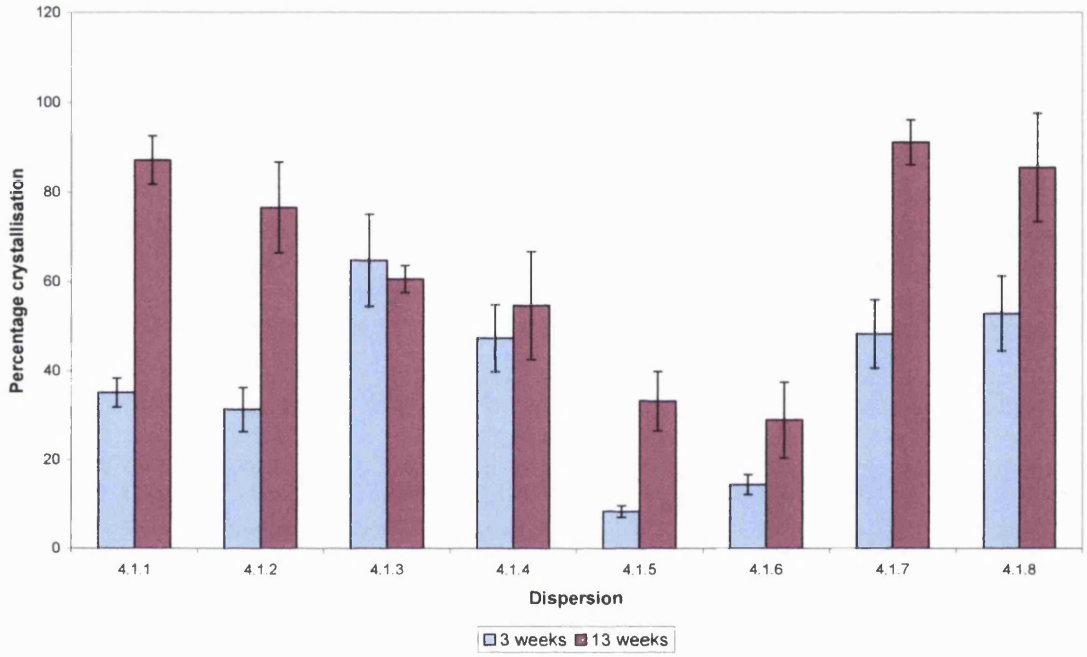

Figure 4.11 The percentage crystallisation of griseofulvin dispersions stored at 40 °C and 0% RH for 13 weeks.

After 3 weeks of storage at 40 °C 0% RH, Dispersion 4.1.5 and Dispersion 4.1.6 (both PHPMA) contained considerably less crystalline material than the other dispersions (Figure 4.11). This suggested that the improvement in stability afforded by the addition of PHPMA was temperature dependent. This was in many ways unsurprising as the T_g of Dispersion 4.1.5 was shown to be 97.9 °C, therefore assuming that the 50 °C rule holds true (Hancock *et al.*, 1995), at 40 °C there would be far less molecular mobility than at 50 °C. The addition of PAA (Dispersion 4.1.3 and Dispersion 4.1.4) and sucrose (Dispersion 4.1.7 and Dispersion 4.1.8) was equally as damaging at 40 °C as at 50 °C, as whilst 35.1% and 31.2% of the griseofulvin in Dispersion 4.1.1 and Dispersion 4.1.2 (binary) had crystallised after 3 weeks griseofulvin in Dispersion 4.1.3 and Dispersion 4.1.4 which both contained PAA.

After 13 weeks storage at 40 °C, 0% RH, near complete crystallisation of Dispersion 4.1.7 and Dispersion 4.1.8 (containing sucrose) was seen (91.3% and 85.5% griseofulvin crystallisation respectively), whilst the binary PVP dispersions (Dispersion 4.1.1 and Dispersion 4.1.2) appeared to be slightly more stable with 87.2% and 76.5% crystallisation respectively, though this difference was not statistically significant (Student's *t*-test; $p < 0.05$). The dispersions containing PAA (Dispersion 4.1.3 and Dispersion 4.1.4) displayed slightly more amorphous stability though the amount of crystallised material present was not significantly different from the amount in Dispersion 4.1.1 and Dispersion 4.1.2 (binary dispersions). This suggested that under these conditions PAA may have helped stabilise the griseofulvin but that this was a temperature dependent effect. At 50 °C the level of griseofulvin crystallisation in Dispersion 4.1.3 and Dispersion 4.1.4 was greater than that of Dispersion 4.1.1 and Dispersion 4.1.2. Moreover, the level of crystalline material after 13 weeks was very much the same as after 3 weeks, suggesting that there was an initial crystallisation after which there was very little crystal growth. The PHPMA dispersions (Dispersion 4.1.5 and Dispersion 4.1.6) were again the most stable and only 33.2% of the griseofulvin in Dispersion 4.1.5 and 28.9% in Dispersion 4.1.6 had crystallised after 13 weeks.

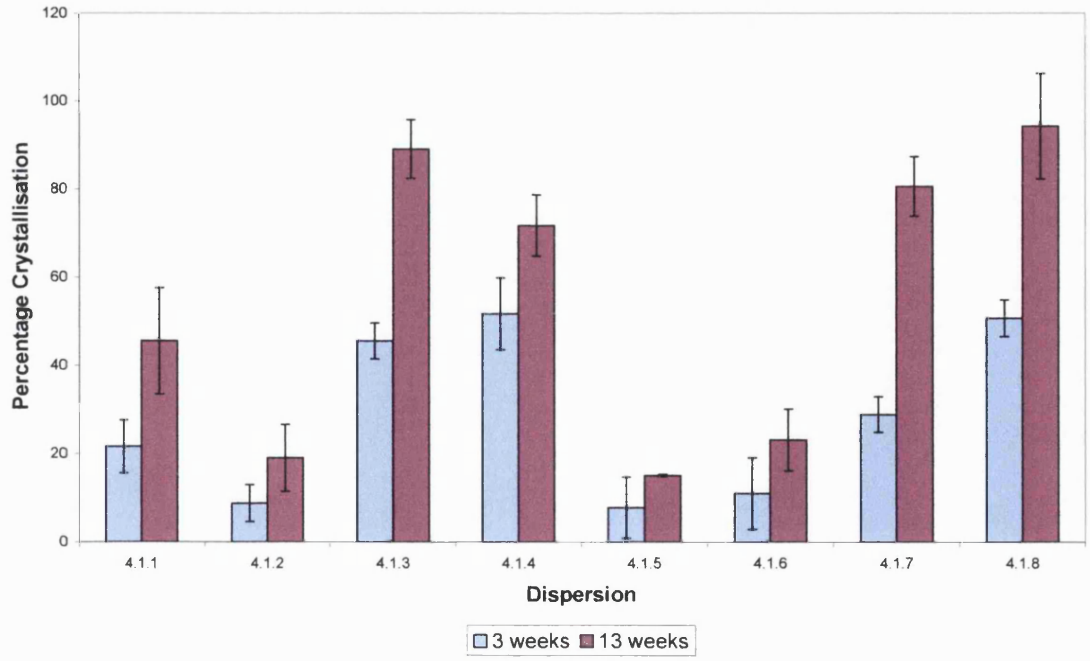

Figure 4.12 The percentage crystallisation of griseofulvin dispersions stored at room temperature and 0% RH for 13 weeks.

When stored at room temperature (20 °C ± 3 °C), 0% RH, the tertiary dispersions containing PAA (Dispersion 4.1.3 and Dispersion 4.1.4) and sucrose (Dispersion 4.1.7 and Dispersion 4.1.8) all displayed greater levels of crystallisation than the binary dispersions; Dispersion 4.1.1 and Dispersion 4.1.2. Dispersion 4.1.5 and Dispersion 4.1.6 (containing PHPMA) again displayed an improved level of stability after both 3 and 13 weeks, though for Dispersion 4.1.6, this was not significantly different from the binary PVP dispersion; Dispersion 4.1.2 (Figure 4.12).

As was seen with the results at 50 °C and 0% RH, when the sucrose dispersions were stored at room temperature, Dispersion 4.1.8 which had the higher level of both PVP and sucrose and also the higher T_g unexpectedly showed a higher level of crystalline griseofulvin (though this was not a significant difference, $p < 0.05$). 94.3% of the griseofulvin crystallised in Dispersion 4.1.8 whilst 80.6% of the griseofulvin in Dispersion 4.1.7 had crystallised after 13 weeks (Figure 4.12). This was also seen with the PHPMA dispersions (Dispersion 4.1.5 and Dispersion 4.1.6), though again this was not significant ($p < 0.05$). After 3 weeks of storage at room temperature a larger percentage of the griseofulvin in Dispersion 4.1.4 (PAA) had crystallised than in Dispersion 4.1.3 (PAA) but after 13 weeks these revert to the expected order of percentage crystallisation.

Overall this may be due to the presence of residual solvent, most likely water, plasticising the dispersions, as has been previously suggested. The presence of water would be particularly detrimental when the dispersions were stored at room temperature as the water was least likely to evaporate at this low temperature.

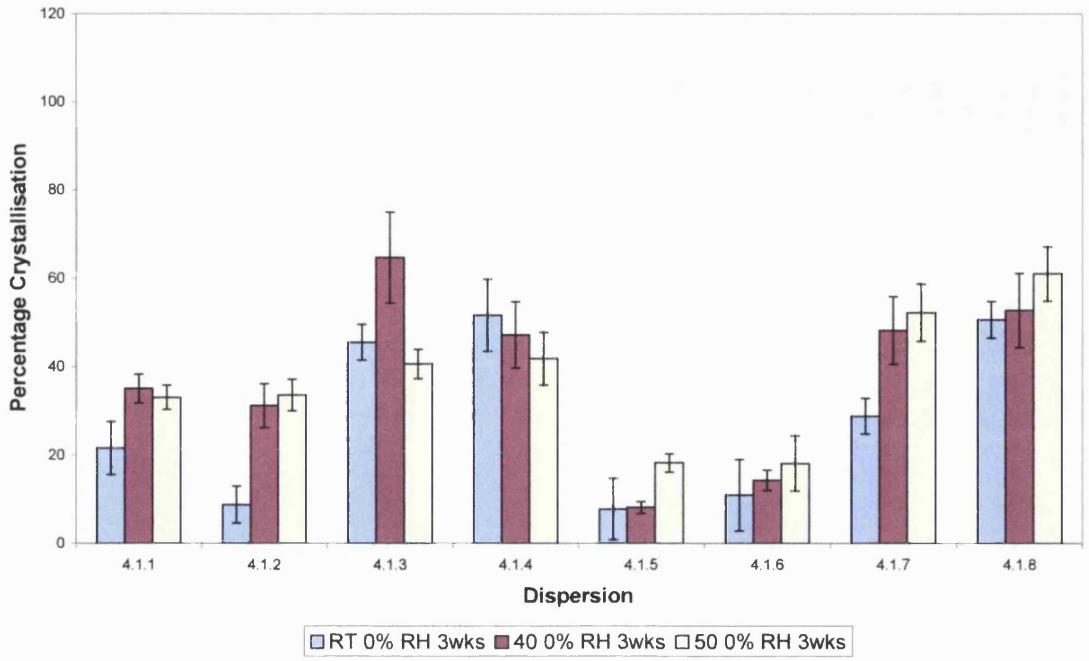

Figure 4.13 The percentage crystallisation of griseofulvin dispersions after 3 weeks when stored under a variety of temperatures at 0% RH.

Figure 4.13 shows the results of crystallisation after 3 weeks of storage at all temperatures and 0% RH. Most of the dispersions followed the expected pattern that increasing temperature produced an increased level of crystallisation. The main exception to this trend was Dispersion 4.1.3 (PAA), in which the highest rate of crystallisation was seen at 40 °C with 64.8% of the griseofulvin having crystallised at that temperature compared with 45.6% having crystallised whilst being stored at room temperature and 40.9% at 50 °C. It was possible that samples retained more water at 40 °C and at room temperature when compared with the samples stored at 50 °C and that at 40 °C there was initially both high levels of solvent and an elevated temperature. Whilst not significantly different, the XRPD results for Dispersion 4.1.4 (PAA) suggest that was the storage temperature increased the amount of griseofulvin that crystallised from the dispersion decreased. This may be due to residual solvent effects.

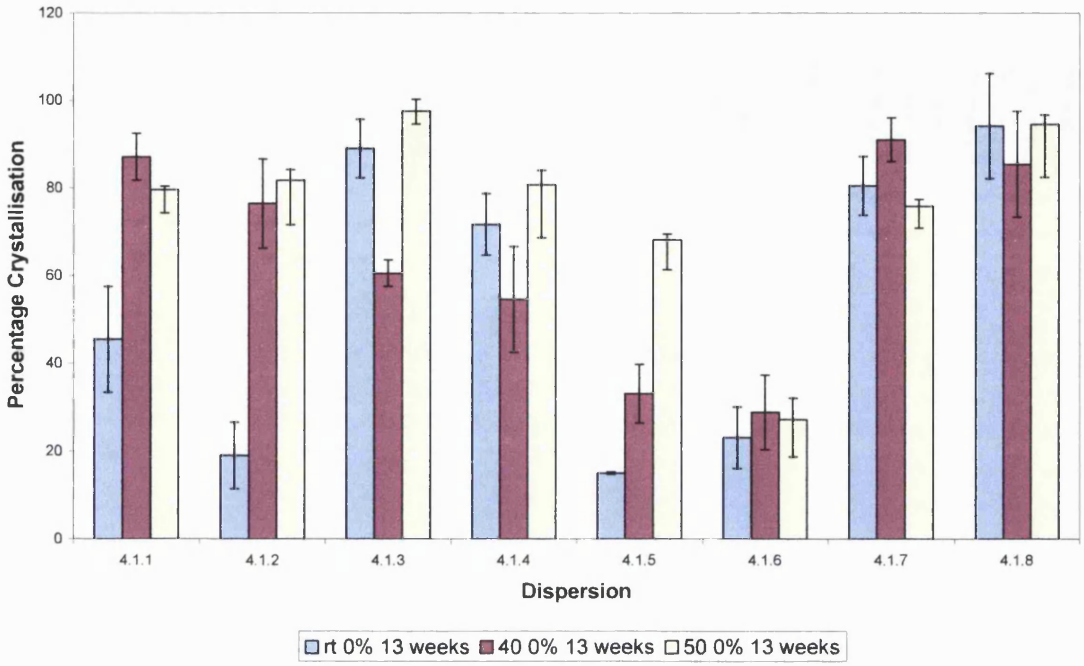

Figure 4.14 The percentage crystallisation of griseofulvin dispersions after 13 weeks when stored under a variety of temperatures at 0% RH.

It was expected that stability and temperature would be directly related and that as the storage temperature increased, the amount of crystalline material observed would similarly increase. After 13 weeks of storage the percentage of griseofulvin that had crystallised in each of the dispersions could not be related to the storage temperature in most cases (Figure 4.14). In Dispersion 4.1.1 (binary) the level of crystallisation when the sample was stored at 40 °C and 50 °C were similar. When the sample was stored at room temperature considerably less griseofulvin crystallisation was observed. A similar trend was seen in Dispersion 4.1.2 (binary) with markedly higher levels of crystalline material being present in the dispersions stored at 40 °C and 50 °C.

After 13 weeks of storage at 40 °C, Dispersion 4.1.3 (PAA) no longer had a higher level of crystallisation than when stored at room temperature or 50 °C. As discussed previously there was very little difference between the level of crystalline material after 3 weeks and after 13 weeks. This suggests an initial rapid crystallisation followed by an extremely slow rate of crystal growth. The results for Dispersion 4.1.4 (PAA) were similar to those for Dispersion 4.1.3 (PAA). The sample stored at 40 °C had a lower level of crystalline griseofulvin after 13 weeks of storage than the samples stored at room temperature or 50 °C. Given that the T_g of Dispersion 4.1.3 was 90.4 °C and the

T_g of Dispersion 4.1.4. was 112.6 °C one would expect that if the storage temperature and T_g were the only two variables affecting crystallisation these two samples would display different temperature dependencies as well as a constantly increasing level of crystallisation. This again suggests that a solvent, most likely water, was increasing the rate of crystallisation.

Dispersion 4.1.5 (PHPMA) showed the expected correlation of increased storage temperature resulting in an increased level of crystalline griseofulvin, and for each temperature increase the percentage of crystalline material slightly more than doubled, from 15.1% (30 °C) to 33.2% (40 °C) and finally to 68.2% (50 °C). In contrast, after 13 weeks the amount of crystalline griseofulvin present in Dispersion 4.1.6 (PHPMA) remained relatively static across the temperature ranges. Once again it was possible that at room temperature less of the residual solvent was driven off and that increased the rate at which the crystallisation process took place. There was also the possibility that unlike the other samples, amorphous griseofulvin was relatively stable in this dispersion and under these conditions the maximal level of crystalline material has been reached after 13 weeks.

The amount of crystalline griseofulvin in Dispersion 4.1.7 (sucrose) after 13 weeks was highest at the storage temperature 40 °C (91.1%), with the percentage of crystalline griseofulvin at room temperature (80.6%) and at 50 °C (75%) being quite similar. This makes it quite likely that the amorphous stability of the dispersion was more affected by solvent at room temperature, and more affected by temperature at 50 °C. The pattern of griseofulvin crystallisation across the different temperatures for Dispersion 4.1.8 (sucrose) was the reverse of that of Dispersion 4.1.7. When stored at 40 °C less of the griseofulvin crystallised (85.5%) than at room temperature (94.3%) or at 50 °C (94.6%), though it is important to note that the values are not significantly different ($p < 0.05$). It was highly likely that nearly all the griseofulvin in this dispersion would have crystallised regardless of storage conditions.

4.3.6 X-RAY POWDER DIFFRACTION RESULTS FOR DISPERSION SET ONE WHEN STORED AT 30% RH

The stability of the amorphous griseofulvin dispersions when stored at 30% RH was also examined to determine the effect of low levels of moisture on the amorphous

stability of the dispersions. Samples of each of the dispersions were stored at room temperature (20 °C ±3 °C), 30% RH and 50 °C, 30% RH.

After the first 3 weeks of storage at 50 °C, 30% RH, both Dispersion 4.1.3 (PAA) and Dispersion 4.1.7 (sucrose) exhibited more crystallisation than Dispersion 4.1.1 (binary). 32.7% of the griseofulvin in Dispersion 4.1.1 had crystallised. The addition of sucrose in Dispersion 4.1.7 resulted in a slightly detrimental effect and 43.1% of the griseofulvin crystallised. 81.7% of the griseofulvin in Dispersion 4.1.3 had crystallised, suggesting that the addition of PAA was detrimental to stability. The addition of PHPMA had a slightly beneficial effect and only 26.6% of the griseofulvin in Dispersion 4.1.5 had crystallised.

After 13 weeks there was complete griseofulvin crystallisation in all the samples that contained 60% griseofulvin by weight. This would suggest that any benefit derived from the addition of a second excipient is most likely to be dependent on the storage conditions; there may not actually be an intrinsic increase in amorphous stability.

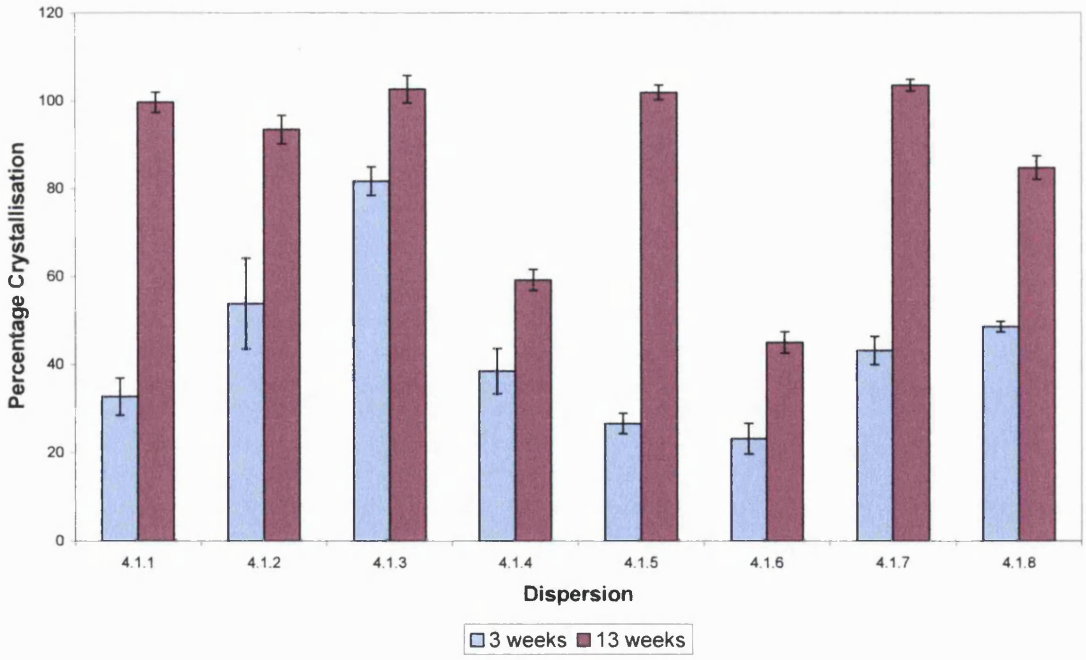

Figure 4.15 The percentage crystallisation of griseofulvin dispersions stored at 50 °C and 30% RH for 13 weeks.

When griseofulvin only made up 40% of each dispersion's weight there were more obvious differences between the samples, with the addition of either PAA or PHPMA

increasing amorphous stability. After 3 weeks 53.9% of the griseofulvin in Dispersion 4.1.2 (binary) had crystallised which was marginally more than in Dispersion 4.1.8 (sucrose) in which 48.6% of the griseofulvin crystallised. Dispersion 4.1.4 (PAA) was more stable still with 38.5% crystallisation, whilst only 23.2% of the griseofulvin in Dispersion 4.1.6 (PHPMA) had crystallised. After 13 weeks nearly all the griseofulvin in Dispersion 4.1.2 had crystallised (93.5%). As was observed after 3 weeks, Dispersion 4.1.8 (containing sucrose) contained less crystalline griseofulvin (84.7%) and Dispersion 4.1.4 (PAA) was more stable still with only 59.2% of the griseofulvin having crystallised. In Dispersion 4.1.6 less than half the griseofulvin (45.0%) had crystallised after 13 weeks of storage.

Given that in Dispersion 4.1.1 all the griseofulvin crystallised it was expected that all the griseofulvin in Dispersion 4.1.2 would also crystallise. Whether the same can be said of the other dispersions was less clear. The original hypothesis was that an interaction between PVP and the secondary excipient (PAA, PHPMA or sucrose) along with an interaction between the secondary excipient and griseofulvin would stabilise griseofulvin in the amorphous form. Whilst this may be the case, it may be that different levels of water uptake during storage could have caused different rates of crystallisation and that this would produce the same result. This may be due to the amount of PVP present in the dispersion, as PVP is well known for being very hygroscopic.

Since the stability experiments were performed under slightly humid conditions, it was felt that water uptake and plasticisation could be the cause of the reduction in stability. Figure 4.16 shows the effect that this slight change in RH had on stability. Relatively small amounts of water can have a large plasticising effect as water has a very T_g, the value of which is generally quoted as being around 136 K or around -137 °C (Angell, 2004). Owing to their very nature, highly water soluble excipients tend to be very hygroscopic. Therefore slight changes in RH will always be an issue when one is trying to produce stable dispersions.

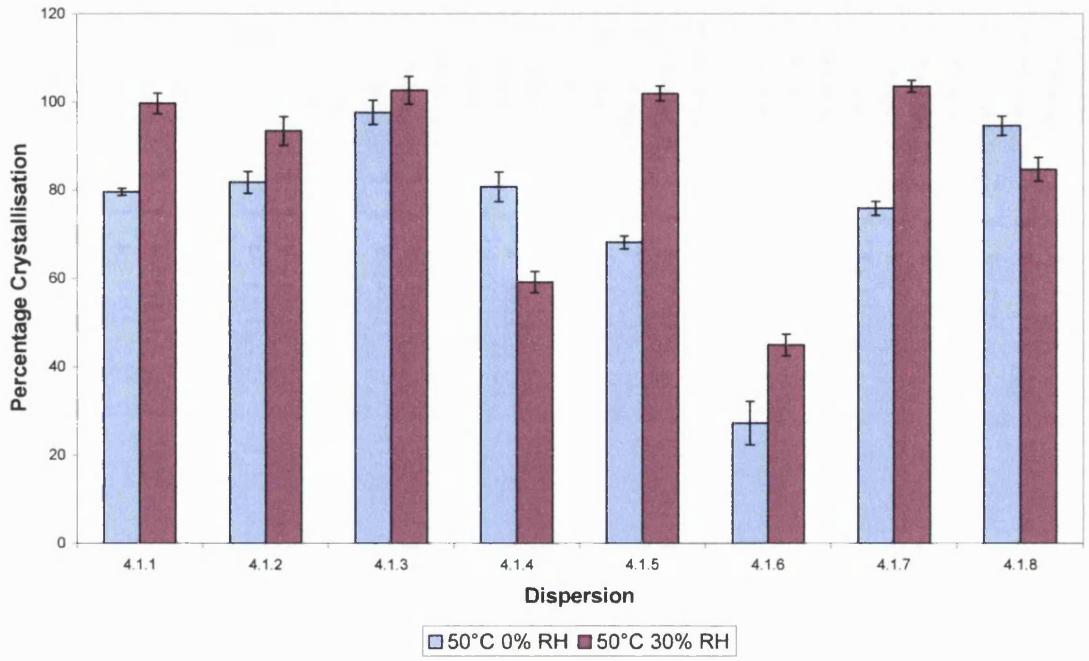

Figure 4.16 The percentage crystallisation of griseofulvin dispersions stored at 50 °C under 0% RH and 30% RH for 13 weeks.

The addition of a secondary excipient may retard the uptake of water by PVP. When combined with the decrease in the overall concentration of PVP this might also reduce the hygroscopy of the dispersion resulting in less plasticisation and hence a lower level of crystallisation. Work performed on dispersions containing 10% and 20% reserpine in PVP in which the dispersions were stored at 20 °C and 50% RH showed that both uptake of water and T_g displayed a level after one day of exposure to 50% RH which did not alter over time. The equilibrium water content in the dispersions was lower than in pure PVP, around 17% for the dispersion and 21% for PVP. Furthermore the T_g proved to be 10 °C higher for the dispersion than in pure PVP. This was despite the low T_g of the drug (Jans-Frontini and Mielck, 1996). Indomethacin, ursodeoxycholic acid and indapamide dispersions with PVP have also been shown to exhibit a deviation from the expected water uptake isotherms. When the drug made up 60% of the dispersion the expected water uptake decreased by 70%. This was based on the components being tested separately and was attributed to interactions between the drugs and PVP (Crowley and Zografi, 2002). When paracetamol-PVP dispersions were compared with paracetamol-PAA not only was PAA more effective at stabilising paracetamol in the amorphous form the PAA dispersions proved to take up less water (Miyazaki *et al.*, 2004).

The stability results from storage at room temperature (20 °C ± 3 °C), 30% RH were markedly different from those at 50 °C 30% RH (Figure 4.17). Indeed, these results were more akin to those from the stability studies performed at 0% RH. After 3 weeks of storage there was very little griseofulvin crystallisation of the binary Dispersion 4.1.1 and Dispersion 4.1.2 (both binary dispersions; 23.1% and 11.5% respectively). Dispersion 4.1.3 and Dispersion 4.1.4 (both PAA) displayed considerably higher levels of griseofulvin crystallisation (59.7% and 51.8% respectively) after 3 weeks. The PHPMA dispersions showed very little change, with 7.2% of the griseofulvin in Dispersion 4.1.5 having crystallised and only 3.7% of the griseofulvin in Dispersion 4.1.6 having crystallised. As with the results from the 0% RH storage conditions, a higher percentage of the griseofulvin in Dispersion 4.1.8 (41.8%) had crystallised than in Dispersion 4.1.7 (35.8%) though this difference was not significant.

After 13 weeks of storage, Dispersion 4.1.1 (binary) proved to have a lower level of crystallisation (64.3%) than either Dispersion 4.1.3 (PAA; 100.0% crystallisation of griseofulvin) or Dispersion 4.1.7 (sucrose; 89.9% crystallisation of griseofulvin) however it was felt that griseofulvin in all these dispersions would have fully crystallised on further storage as the dispersions stored at 50 °C 30% RH did.

Of the dispersions containing PVP as the major component, Dispersion 4.1.6 (PHPMA) was the most stable, with only 22.1% of the griseofulvin having crystallised after 13 weeks. This compares favorably with Dispersion 4.1.2 (binary) in which 30.4% of the griseofulvin had crystallised. Within Dispersion 4.1.4 (PAA), 80.4% of the griseofulvin had crystallised after 13 weeks, which was considerably more than the 59.2% of the griseofulvin that had crystallised after 13 weeks storage at 50 °C and 30% RH. Similarly after 13 weeks, marginally more of the griseofulvin in Dispersion 4.1.8 (sucrose) had crystallised after 13 weeks storage at room temperature and 30% RH (89.9%) than after 13 weeks storage at 50 °C 30% RH in which 84.7% of the griseofulvin had crystallised.

This paradox of higher temperatures failing to induce an increased amount of crystallisation is difficult to explain. Initially it was suspected that residual solvent levels may have affected the results, as they did for dispersions stored under 0% RH. Figure 4.18 shows a sample vapour sorption trace of PVP exposed to a DVS cycle of

0% RH then, 30% RH and back to 0% RH. In this experiment the dry PVP absorbed around 11% of its own weight in water, a value which was not dissimilar to the literature value (Rowe *et al.*, 2003). As the residual solvent present in the dispersions was between 1.9-2.5%, residual solvents were unlikely to have made a large contribution to the overall solvent content of the dispersions and the samples stored at room temperature and 30% RH probably contained a similar level of water to those samples stored at 50 °C, 30% RH.

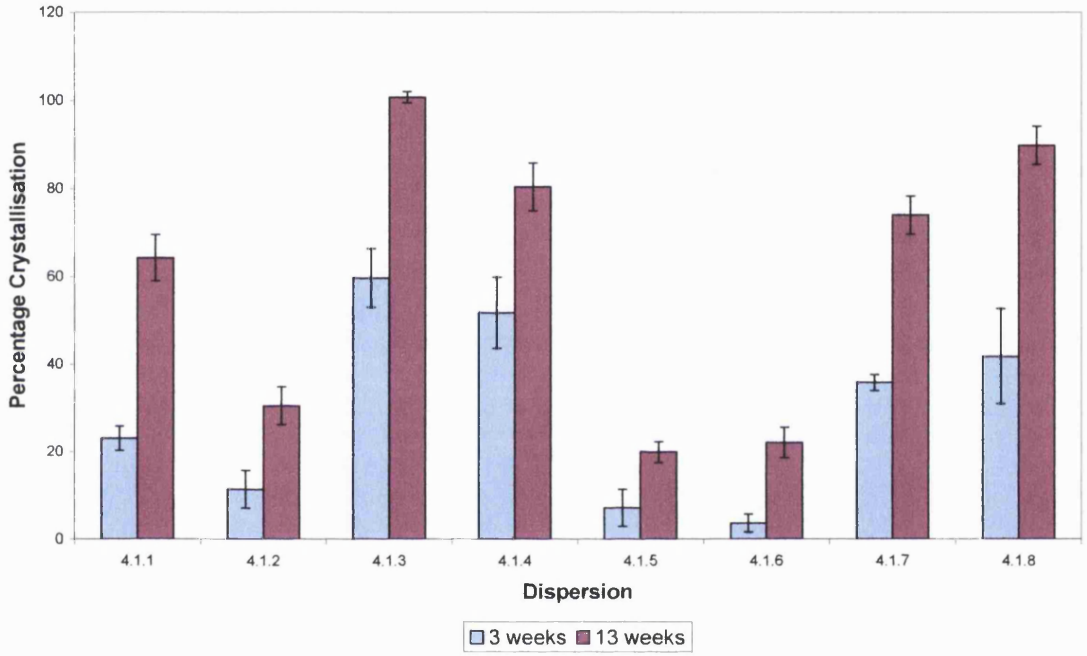

Figure 4.17 The percentage crystallisation of griseofulvin dispersions stored at room temperature and 30% RH for 13 weeks.

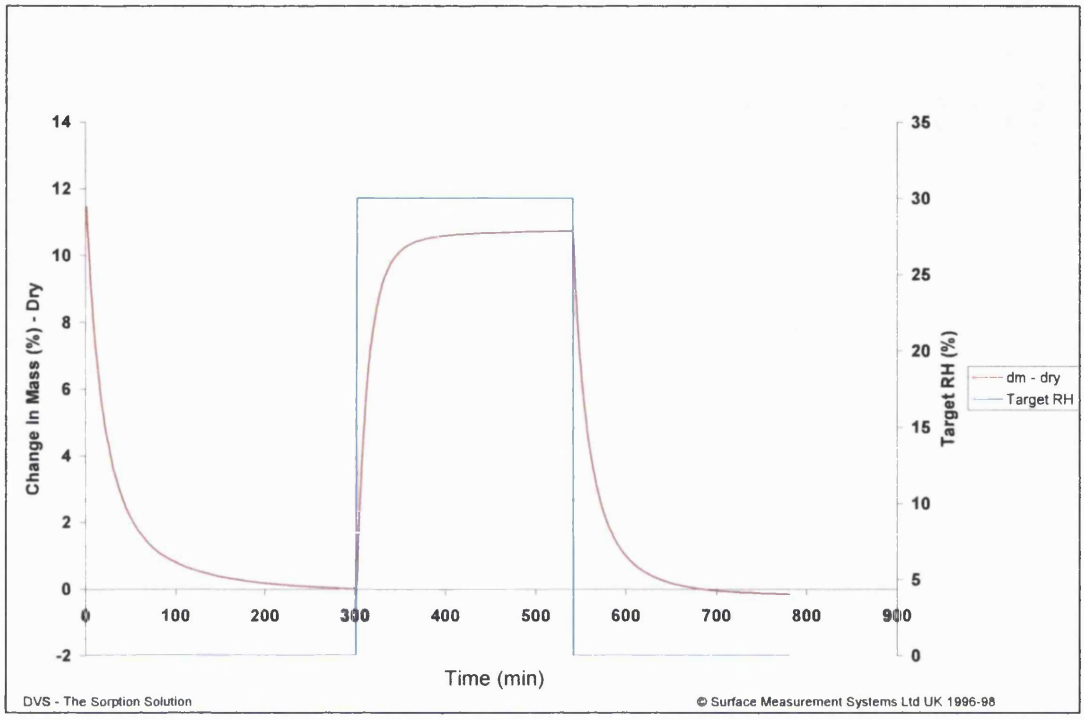

Figure 4.18 A DVS trace showing the percentage change in mass of PVP under a relative humidity cycle of 0%-30%-0% RH.

Amorphous stability cannot be linked to the T_g of the individual dispersions. Dispersion 4.1.3 (PAA), Dispersion 4.1.5 (PHPMA) and Dispersion 4.1.7 (sucrose) all had a similar T_g but crystallised to a different degree when stored at 30% RH. This was also seen when the dispersions were all stored at 0% RH. Differences in water sorption were unlikely to be a major contributing factor. PAA and PVP are known to interact very strongly (Florence and Attwood, 1998). It may be that this interaction occurred at the expense of the interaction between PAA and griseofulvin. This would have led to discrete areas of the polymer complex and discrete areas of drug. It is also possible that if these discrete areas were small enough, a single T_g would have been observed by DSC. Furthermore PAA interacts reasonably strongly with itself, thus it may also be forming a discrete segment of the dispersion. Irrespective of the conditions thus far PHPMA has shown promise as a bridging excipient to improve the amorphous stability of griseofulvin in PVP.

4.3.7 The Stability of Dispersion Set Two

Through the study of the stability of the dispersions consisting of griseofulvin, PVP and a second excipient which have been presented thus far it has been observed that the addition of a relatively small amount of a secondary excipient can have a profound

effect on the amorphous stability of the griseofulvin in the dispersion. Only one of these excipients, PHPMA, consistently showed any real benefit in stabilising the amorphous griseofulvin. It was decided that the effect that increasing the amount of PHPMA had on stability would be investigated.

Three dispersions were prepared, each of which contained 60% w/w griseofulvin. The concentration of griseofulvin was kept high in order to maximise the differences that would be observed between the dispersions. Dispersion 4.1.1a (binary) and Dispersion 4.1.5a (10% w/w PHPMA) were the same as Dispersion 4.1.1 (binary) and Dispersion 4.1.5 (10% w/w PHPMA). Dispersion 4.2.5 contained 20% w/w PVP and 20% w/w PHPMA. The results of XRPD studies on these griseofulvin dispersions have been presented in Figure 4.19.

The presence of PHPMA again reduced the overall level of crystallisation, as was seen in Dispersion 4.1.5a (10% w/w PHPMA) and Dispersion 4.2.5 (20% w/w PHPMA). After 7 weeks of storage 80.0% of the griseofulvin in Dispersion 4.1.1a (containing griseofulvin and PVP) had crystallised. This was remarkably similar to the amount seen in Dispersion 4.1.1 after 13 weeks (79.6%), however after 3 weeks considerably more crystalline material was seen in Dispersion 4.1.1a (54.7%) than was seen in Dispersion 4.1.1 (33.1%). This was most likely due to differences in residual solvent.

In comparison to the binary dispersion (Dispersion 4.1.1a), after 7 weeks only 57.1% of the griseofulvin in Dispersion 4.1.5a (10% w/w PHPMA) had crystallised and in Dispersion 4.2.5 (20% w/w PHPMA) only 45.7% had crystallised. Dispersion 4.1.1a contained crystalline griseofulvin after the first week of storage (18.9%). Crystalline griseofulvin was not detected in Dispersion 4.1.5a until week 3 (3.6%) and Dispersion 4.2.5 did not begin to crystallise until week 4 (3.1%). The increased lag time prior to crystallisation and the reduced level of crystallisation was likely to be due to an increased level of hydrogen bonding. PHPMA can interact with both PVP and griseofulvin through hydrogen bonding. Without this "bridging" action there would be relatively little in inter-molecular interactions and one would expect the rate of crystal nucleation to be more rapid.

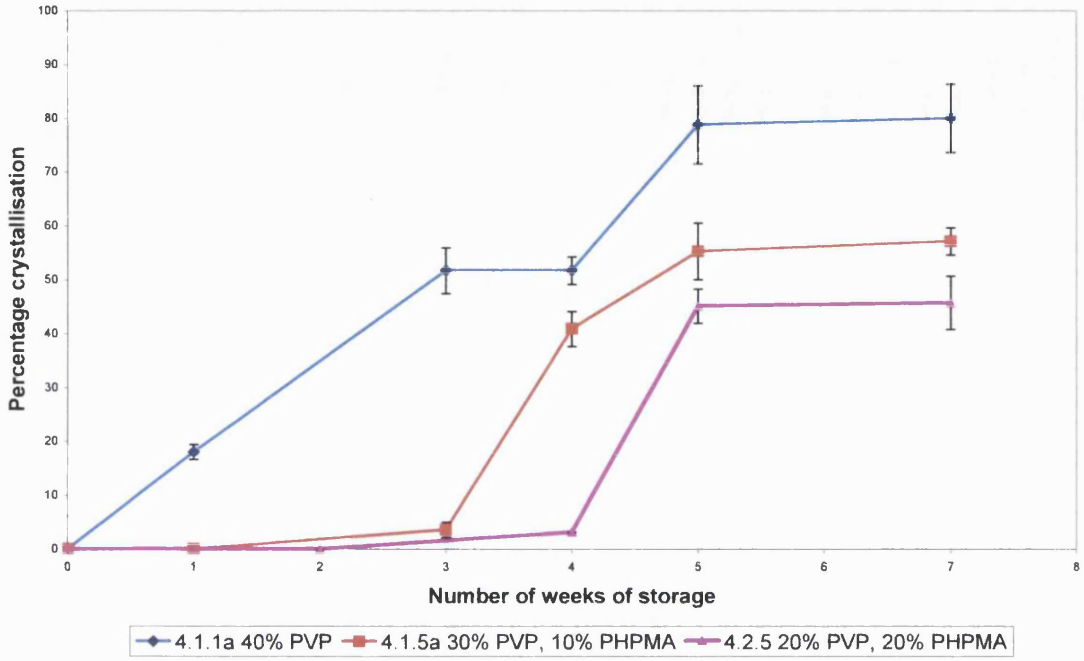

Figure 4.19 The percentage crystallisation of griseofulvin dispersions containing PVP with varying amounts of PHPMA.

Table 4.6 contains the T_g of the three components used in this experiment. A "heat-cool-heat" DSC experiment was used to remove water or other solvents prior to the location of the T_g. The data listed in Table 4.6 suggest that PVP has by far the highest T_g and this is due in part to the large inflexible pyrrolidone side chain, which limits molecular mobility. PHPMA, has a more flexible side chain than PVP and therefore has a lower T_g. The T_g of griseofulvin is remarkably high for a small molecule.

Dispersion Constituent	**T_g (°C)**
Griseofulvin	92.1 ±1.3
PVP	168.6 ±0.1
PHPMA	75.4 ±0.6

Table 4.6 The T_g of the constituents of dispersions that have been prepared in this section.

These values were used to calculate theoretical T_g of each of the dispersions and were then compared with the experimental T_g. The results are shown in Table 4.7a and 4.7b. In Section 4.1 there was a discussion of the two mechanisms by which stabilisation of a drug in the amorphous form in a solid dispersion with polymeric excipients is thought to

occur. Stabilisation can be due to an increased T_g, or can be due to specific interactions between the polymer and the drug.

Typically dispersions or complexes of polymers which do interact such as PVP and PAA (Lau and Mi, 2002) or PVP and PHPMA (Kuo *et al.*, 2004) display an elevated T_g over the theoretical T_g. In Table 4.7a the theoretical T_g has been calculated based on a weighted average of the T_g of the individual components. This has been compared with the actual T_g. It is interesting to note that the actual T_g was a fairly consistent percentage of the theoretical T_g. Residual solvents reducing T_g can be an issue and so the theoretical T_g was recalculated to take into account the TGA results with the assumption that the weight loss in the TGA was entirely due to water loss. Even with the recalculation the actual T_g remained slightly lower than the theoretical T_g (Table 4.7b).

The T_g for Dispersion 4.2.5 (20% w/w PHPMA) was considerably lower than that of Dispersion 4.1.1a (binary dispersion). Dispersion 4.1.5a (10% w/w PHPMA) has a T_g which lies in between these two extremes. At the same time, Dispersion 4.2.5 was more stable than Dispersion 4.1.5a which was in turn more stable than Dispersion 4.1.1a. The rational for adding PHPMA to the dispersion matrix was that the PHPMA would act as a proton donating bridge between PVP and griseofulvin. As the level of interactions was increased (by increasing the amount of PHPMA) the amorphous stability of the griseofulvin should have increased. Whilst the increase in amorphous stability was observed, the elevated T_g that was expected to result from the increased level of interactions was not observed.

Dispersion	**Actual T_g (°C)**	**Theoretical T_g (°C)**	**Percentage actual T_g was of theoretical T_g**
4.1.1a	109.5 ±1.2	122.7	89.2
4.1.5a	98.3 ±1.1	113.4	86.6
4.2.5	94.6 ±0.5	104.0	91.0

Table 4.7a Theoretical T_g versus T_g for Dispersion 4.1.1a (binary dispersion), Dispersion 4.1.5a (containing 10% PHPMA) and Dispersion 4.2.5 (containing 20% PHPMA).

Dispersion	Percentage residual solvent	Theoretical T_g (°C)	Percentage actual T_g was of theoretical T_g
4.1.1a	2.7 ± 0.2	115.7	94.7
4.1.5a	2.2 ± 0.4	107.9	91.1
4.2.5	2.3 ± 0.7	98.5	96.1

Table 4.7b Residual solvent content and theoretical T_g versus actual T_g for Dispersion 4.1.1a (binary dispersion), Dispersion 4.1.5a (containing 10% PHPMA) and Dispersion 4.2.5 (containing 20% PHPMA) when the calculations take into account the presence of water.

4.3.8 The Stability of Dispersion Set Three - The Effect of the Order of Addition

In Chapter 3 the order in which Eudragit S, PVP and indomethacin were dissolved in the feed solution prior to spray drying affected the pH at which dissolution of the dispersion microparticles occurred. It was hypothesised that the different components of the dispersion were interacting in solution prior to spray drying and that the nature of these interactions changed depending on the order in which the components of the dispersion were dissolved in the feed solution. It was decided that the same could be true for dispersions under investigation in this chapter and thus experiments were performed to try and improve the level of stability by examining the effect that changing the order of addition had on the stability of the dispersions.

This third set of dispersions was stored at 50 °C and 0 % RH, with XRPD being performed weekly. Figure 4.20 shows the percentage crystallisation of the griseofulvin during storage as was detected by XRPD. The use of PHPMA in the dispersion increased the amorphous stability of griseofulvin irrespective of the order in which the components of the dispersion were dissolved. However, the level by which crystallisation was retarded appeared to be dependent on this order. The most stable dispersion was that in which griseofulvin and PVP were dissolved together prior to the addition of the PHPMA (Dispersion 4.1.5b). This was the order of addition to the feed solution that had been used to prepare the previous dispersions. When the dispersions were tested for crystalline material immediately after spray drying (within 30 min), all

were found to be amorphous with the exception of Dispersion 4.3.5b, which was prepared by dissolving griseofulvin then PHPMA and finally PVP. In this dispersion 12.0% of the griseofulvin was found to be crystalline immediately after spray drying. This was most likely due to the presence of undissolved crystals of griseofulvin in the spray drying feed solution. These crystals could quite easily have been created when the distilled water was added to the solution. This proposal is complemented by the previous solubility experiments that demonstrated just how close to a saturated solution of griseofulvin the feed solutions were. Spray dried particles containing separate phases in combination with the presence of residual solvent or excessively high temperatures could lead to crystallisation. However this is unlikely to be the case as in Section 4.3.1 it proved possible to produce amorphous griseofulvin through spray drying under very similar conditions.

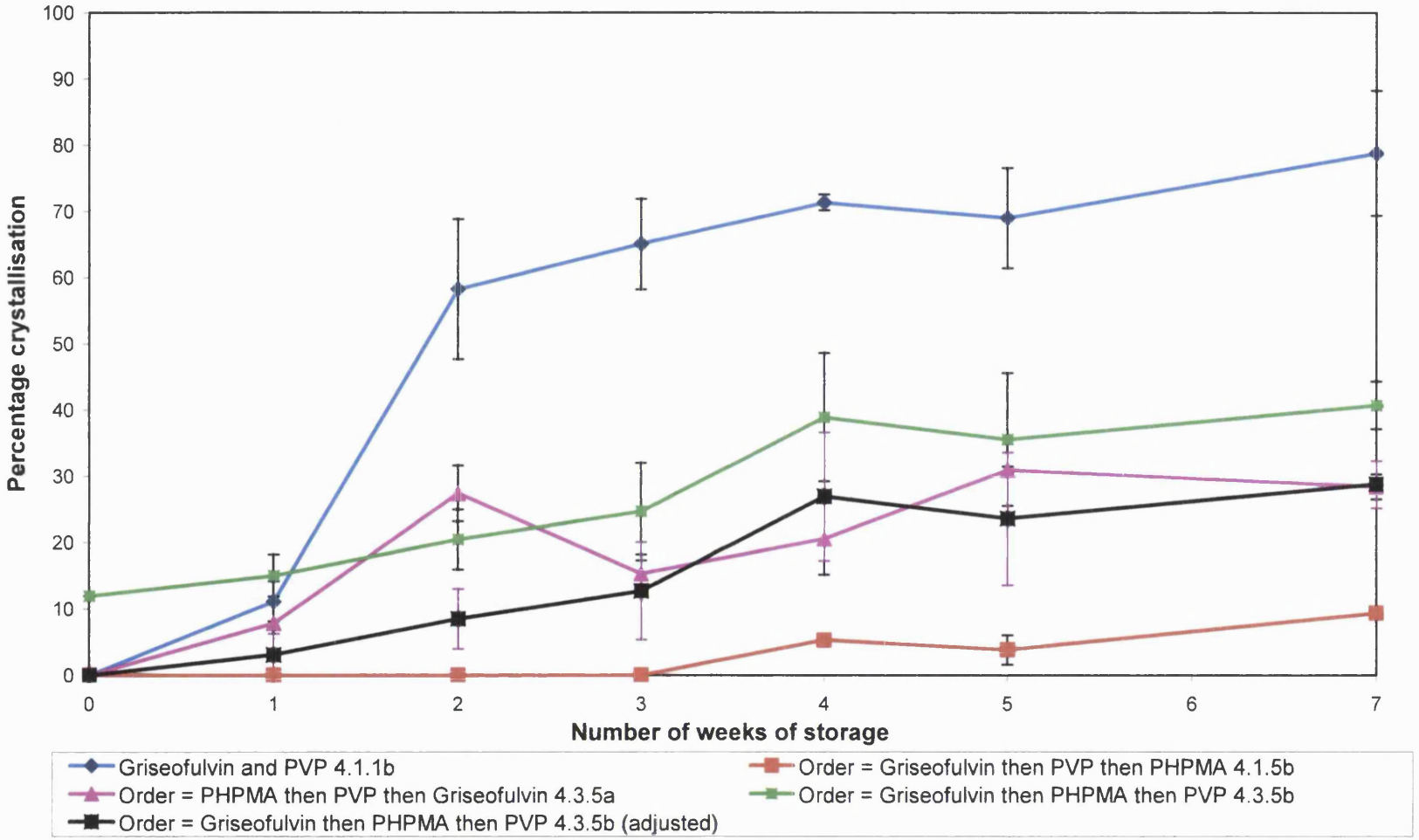

Figure 4.20 The percentage crystallisation of griseofulvin with respect to time from various dispersions containing 60% griseofulvin by weight with PVP and PHPMA.

Crystallisation is generally separated into two main events; crystal nucleation and crystal growth (Mullin, 2001). It was felt that by subtracting the percentage crystallisation present at time zero from the later readings a reasonable comparison could be made. This has also been presented in Figure 4.20. When this was done, the rate of crystallisation becomes almost linear with respect to time, suggesting that it was only crystal growth that contributed to the increase in crystalline material in Dispersion 4.3.5b.

After 7 weeks of storage, 78.8% of the griseofulvin in Dispersion 4.1.1b (containing griseofulvin and PVP) had crystallised. Dispersion 4.1.5b (griseofulvin, PVP and then PHPMA) showed by far the greatest level of retardation of crystallisation with only 9.4% of the griseofulvin having crystallised after 7 weeks. Dispersion 4.3.5a and 4.3.5b had remarkably similar levels of griseofulvin crystallisation after 7 weeks when the pre-existing crystal material was taken into account in Dispersion 4.3.5b. The stability benefit of Dispersion 4.1.5b was most likely due to two main effects. Firstly, having the most ideally mixed composition, and secondly, that PHPMA was interacting with both the PVP and the griseofulvin equally. In effect it was acting as a bridge between the griseofulvin and the PVP holding them together in the amorphous form.

The T_g for each of the dispersions appeared to be essentially the same when DSC experiments were performed (Table 4.8) and this was expected as the content of the dispersions was broadly the same, and the level of interaction (hydrogen bonding) in each dispersion was subsequently expected to be the same.

Dispersion	Tg (°C)
4.1.1b	109.9 ± 1.8
4.1.5b	94.5 ± 0.7
4.3.5a	95.4 ± 1.0
4.3.5b	97.2 ± 2.2

Table 4.8 The T_g for dispersions of griseofulvin with PVP and PHPMA.

As the T_g of each of the different tertiary dispersions was essentially the same and the level of interaction in each of the dispersions was expected to be essentially the same, the reason for the variations in stability must have been due to the nature of the

interactions. It has been suggested that an amorphous product may display a varying degree of disorder and that this may not be separated into discrete levels of disorder. When testing material that is essentially amorphous in nature that also contains areas of short range order (which may act as crystal nuclei) the sample will appear amorphous when XRPD is used. XRPD only detects long range order. Either of these effects may result in a variation in the rate of crystallisation. The residual solvents water and acetone may also be present. Water is known to have a very low T_g (Angell, 2004). It was felt that acetone would have a similarly low T_g. If there were significant variations in solvent content one would expect to see significant variations of the dispersion T_g. Therefore it is most likely that the way in which PHPMA interacts with the other dispersion components is paramount to stability.

Any of the PHPMA sub-units can interact with PVP, griseofulvin or other PHPMA subunits. The stability of a solid dispersion depends on miscibility and the production of a uniform system. Therefore the most stable dispersion should be the one in which PHPMA can interact with both griseofulvin and PVP. It was believed that this was the case in Dispersion 4.1.5b, as both griseofulvin and PVP are present in solution prior to the addition of the PHPMA. Thus, PHPMA was able to interact with both components equally. When PHPMA was mixed with either PVP or griseofulvin before the addition of the final component it was highly likely that one or other interaction predominated which, whilst increasing amorphous stability over the binary solution was unlikely to provide optimum stability.

4.4 CONCLUSIONS

Amorphous griseofulvin, despite having a high T_g, rapidly crystallises even when stored at room temperature, 0% RH. The production of dispersions of griseofulvin in PVP can improve amorphous stability though this will not prevent the griseofulvin from crystallising eventually. The addition of a third component to the dispersion which interacts with both PVP and griseofulvin can improve stability, however care is required as not all additives may be beneficial, and indeed the third component may prove to be detrimental to stability. The order in which each component is added to the spray dryer feed mix can have a profound effect on stability as can the concentration of the components.

Chapter 5

The Stability of Dispersions Containing Clotrimazole and Flavanone

5.1 INTRODUCTION

As with Chapter 4, the work presented in this chapter is also concerned with the effect that a small amount of a tertiary excipient has on the amorphous stability of small molecules dispersed in polyvinylpyrrolidone (PVP). The use of PVP to stabilise drugs in the amorphous form as part of a molecularly dispersed solid dispersion (solid/glass solution) has already been discussed in detail in the preceding chapters. Briefly, holding a poorly water soluble drug in the amorphous form should result in an increased dissolution rate and apparent saturation solubility (Hancock and Parks, 2000). The use of a highly water soluble carrier excipient should also increase the surface area over which dissolution can occur. The hope being that increasing solubility in the GI tract will result in an increase in bioavailability as is seen when drugs are micronised (Chaumeil, 1998; Serajuddin, 1999). As was discussed in Chapters 1 and 4 the use of methods like micronisation or solid dispersions will result in an essentially unchanged active.

There is considerable debate as to the mechanism by which drugs are stabilised in the amorphous form. The stabilising effect may be due to direct interactions between excipients and drug (Yoshioka *et al.*, 1995) or it may only be an anti-plasticising effect in which an overall decrease in molecular mobility is due to the presence of a polymer with a high glass transition temperature (T_g) (Van den Mooter *et al.*, 2000). It most likely to be the case that both mechanisms contribute to stabilising the amorphous state and the mechanism that is predominant is a function of the specific formulation.

The work presented in Chapter 4 was concerned with increasing the amorphous stability of griseofulvin and PVP dispersions by adding a second excipient which could act as a proton-donating bridge between griseofulvin and PVP. This chapter continues to examine the use of a bridging excipient. Two different small molecules were examined; flavanone and clotrimazole. Flavanone, the structure of which is shown in Figure 5.1, was selected as a further model for drugs that, like griseofulvin, lack proton donating groups. Theoretically it should interact poorly with PVP and hence poor amorphous stability was expected when binary dispersions were prepared. A short term stability study comparing a flavanone and PVP dispersion with flavanone, PVP and poly(2-hydroxypropylmethacrylate) (PHPMA) dispersions was performed. PHPMA was chosen as in Chapter 4 it was shown to be the most effective stabiliser of amorphous griseofulvin dispersed in PVP.

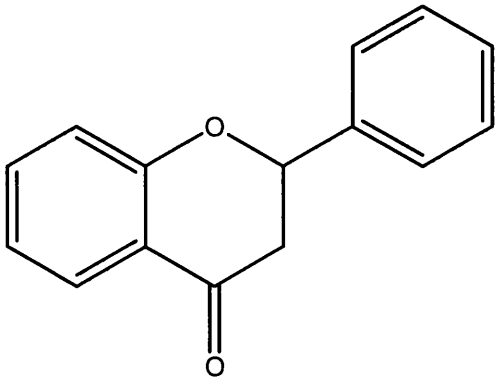

Figure 5.1 The structure of flavanone.

Clotrimazole, the structure of which is shown Figure 5.2, is typically used as a local topical antifungal. Whilst it is generally not used orally there are certain structural similarities between clotrimazole and orally active anti-fungals such as ketoconazole (Figure 5.3) and itraconazole (Figure 5.4). Chief of these are the lack of proton donating groups and the presence of two tertiary amine groups in the imidazol ring. The presence of these tertiary amine groups was one of the major factors behind the investigation of clotrimazole. Since these amines are very weakly basic it seemed there was little chance they would become charged in order to interact, via hydrogen bonding, with the carbonyl group on PVP, as has been seen with secondary amines such as frusemide (Akbuga *et al.*, 1988; Doherty and York 1989). Ketoconazole has been shown to be stable in the amorphous form in PVP and this has been ascribed to an anti-plasticising effect as it does not strongly interact with PVP (Van den Mooter *et al.*, 2000). However this does not preclude the possibility that stability could be improved if there was an increased level of hydrogen bonding.

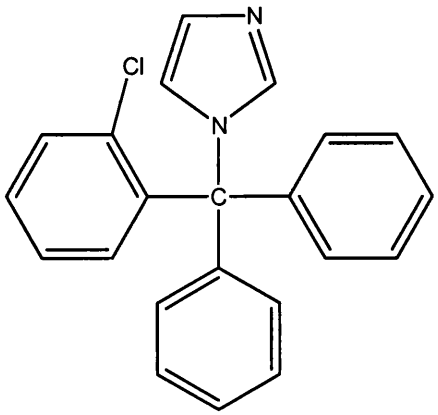

Figure 5.2 The structure of clotrimazole.

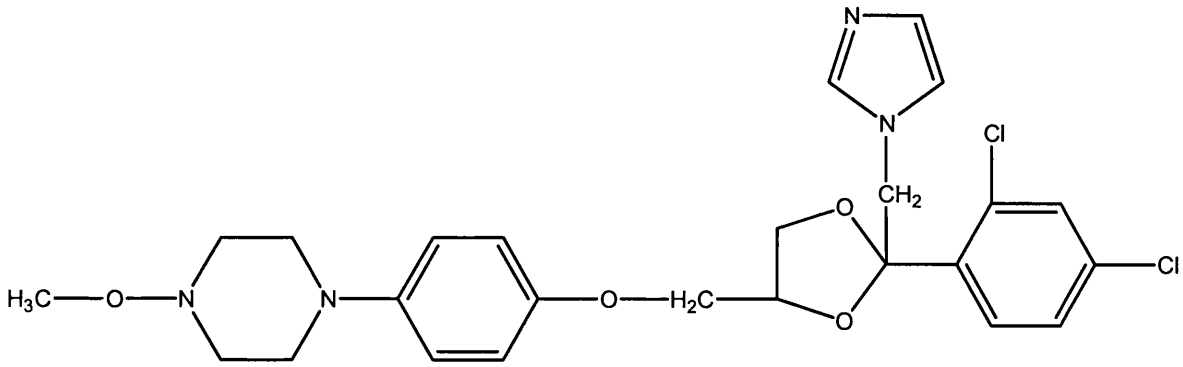

Figure 5.3 The structure of ketoconazle.

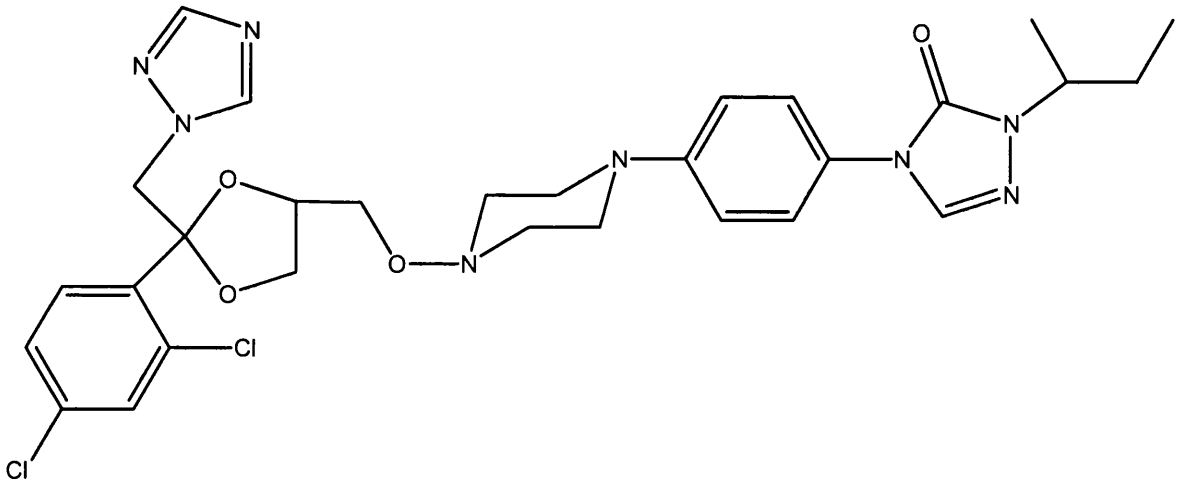

Figure 5.4 The structure of itraconazole.

Solid dispersions of PVP and a second excipient that contained a high concentration of clotrimazole (75% w/w) were initially investigated in order to determine the effect that said secondary excipient would have on amorphous stability. These were compared with binary clotrimazole and PVP dispersions. Similar dispersions containing clotrimazole, PVP and a second excipient with lower concentrations of clotrimazole (40% w/w and 60% w/w) were produced. These dispersions were also compared with binary clotrimazole and PVP dispersions in order to examine the effect that the presence of a second excipient had on amorphous clotrimazole stability. These dispersions were stored under both dry and humid conditions in order to examine the effect moisture had on amorphous stability.

5.2 METHODS

5.2.1 SMALL SCALE SOLUBILITY STUDIES

Small scale solubility studies using the solvents acetone, ethanol and water were performed. Flavanone and clotrimazole (50-100 mg) were added to 1 ml of each solvent. Given the results of the previous chapters the main aim was not to consider absolute solubility but merely to confirm which solvents would be acceptable for the production of spray dryer feed solutions. It was therefore decided that confirmation of dissolution or its absence would be by eye only. The solutions of each of the polymers and sucrose were made up as per Sections 2.2, 3.2.2 and 4.2.2 Small scale combination experiments were then performed in which each of the drug solutions (400 µl) was measured into a 2 ml glass vial. In turn each of the excipient solutions (200 µl) was then added. Finally, the PVP solution (200 µl) was added and any resulting precipitation was recorded.

5.2.2 CHARACTERISATION OF CRYSTALLINE FLAVANONE AND CRYSTALLINE CLOTRIMAZOLE

Flavanone was characterised using X-ray powder diffraction (XRPD) in order to ascertain which (if any) of the X-ray peaks would be useful for the analysis of spray dried dispersions containing flavanone and to provide an area for these peaks which could then be used to calculate the percentage of flavanone crystallisation in the solid dispersion. This was as per Section 2.7 In total 10 scans were performed to produce the peak areas of 100% flavanone. The radiation used was generated by a copper kα filter, with a wavelength of 1.5418 °A at a voltage of 45 kV and current of 30 mA. The samples were scanned over a range of 15-30 °2Θ at a scan rate of 0.5 °2Θ/min. An assessment of the glass transition temperature (T_g) of flavanone using DSC was attempted as per Section 2. however amorphous flavanone proved to be unstable and crystallisation enthalpies were detected during the cooling cycle of "heat-cool-heat" DSC experiments.

Characterisation of crystalline clotrimazole was performed using differential scanning calorimetry (DSC) to determine an accurate onset temperature and enthalpy (ΔH) for the melt. Four DSC scans on samples were performed across the range 30-200 °C at a rate of 10 °C/min as described in Section 2.5. Characterisation of the degree of crystallinity was also performed using XRPD as per Section 2.7.

5.2.3 PRODUCTION AND ANALYSIS OF SPRAY DRIED FLAVANONE DISPERSIONS WITH PVP AND PHPMA

Three dispersions containing flavanone were prepared. Dispersion 5.F.1 was prepared by dissolving accurately weighed Flavanone (2.66 g) in stirred acetone (150 ml) in a 200 ml conical flask. After 2 h distilled water (30 ml) was then added and the solution was allowed to mix for a further 15 min. This was followed by the addition of solid PVP (4 g). Dispersion 5.F.2 was prepared by dissolving Flavanone (2.66 g) in stirred acetone (150 ml) in a 200 ml conical flask. After 2 h distilled water (30 ml) was then added and solution allowed to mix for a further 15 min. This was followed by the addition of solid PVP (3 g). Once the PVP had dissolved (15 min), PHPMA (1 g) was added. The solution was then allowed to mix for a further 2 h before being spray dried. Dispersion 5.F.3 was prepared in the same manner as Dispersion 5.F.2 except that 2 g of PVP and 2 g of PHPMA were used.

The solutions were then spray dried using the SD Niro as described in Section 2.3. An inlet temperature of 60 °C was used with the outlet set at 40 °C (both ±3 °C). The nitrogen flow rates were 20 kg/h and 2 kg/h for the drying and pulverising steps respectively. The feed rate was held at 20% (±2%) of the maximum feed rate, which equated to a feed rate of approximately 3 ml/min. On completion of the spray drying each sample was stored in a 100 ml Durran vessel in a desiccator containing phosphorous pentoxide under vacuum at room temperature (20 °C ±3 °C) for 30 days.

Early experiments had shown that flavanone had a tendency to crystallise upon heating, therefore DSC was ruled out as the method of study. Instead the samples were analysed using XRPD to find the percentage crystallinity. Diffraction patterns were obtained on a Philips PW3710 scanning X-ray powder diffractometer (Philips, Netherlands). The radiation used was generated by a copper kα filter, with a wavelength of 1.5418 °A at a voltage of 45 kV and current of 30 mA. The samples were scanned over a range of 15-30 °2Θ at a scan rate of 0.5 °2Θ/min. The percentage of flavanone that had crystallised was calculated by integrating the peaks found at around 22 °2Θ and at around 24 °2Θ as described in Sections 2.7, 4.2.3 and 4.2.5. The results were then compared with those found for pure flavanone (Section 5.2.2) to provide a percentage crystallisation.

5.2.4 PRODUCTION AND ANALYSIS OF SPRAY DRIED CLOTRIMAZOLE DISPERSIONS CONTAINING CLOTRIMAZOLE 75% W/W

In order to rapidly assess the effect that the addition of a second excipient would have on the crystallisation of clotrimazole from solid dispersions, the dispersions that were produced contained a high concentration of clotrimazole. These dispersions were compared with a clotrimazole and PVP binary dispersion. The contents of the dispersions are listed in Table 5.1. The dispersions were prepared as follows. Clotrimazole (6 g) was accurately weighed and dissolved in stirred ethanol (80 ml) in a 200 ml conical flask for 2 h. PVP (1.5 or 2 g) was then added to the stirred clotrimazole solution and allowed to dissolve for 15 min. Distilled water (20 ml) was then added. The solution was stirred for a further 10 min. A second excipient was then added as a solid, if required (as listed in Table 5.1). The solution was stirred for a further 2 h before being spray dried under nitrogen using the SD Niro Spray Dryer (see Section 2.3). The inlet and outlet temperatures were 65 °C and 45 °C respectively (both ±3 °C) whilst the nitrogen flow rates were 20 kg/h and 2 kg/h. The flow rate of the feed solution was 20% (±2%) of the maximum feed rate (≈ 3 ml/min). DSC was performed on the samples immediately after spray drying to confirm that the samples were amorphous. The samples were then stored in a 100 ml Durran vessels in a desiccator at 0% RH (200 g P_2O_5), under vacuum at room temperature. DSC experiments were performed over the following 60 days to examine the level of crystallisation of the samples. These experiments were simple heating scans from -50 to 150 °C at both 10 °C/min and 200 °C/min with 4 scans being taken at each time point.

Dispersion number	Amount of Clotrimazole (g)	Amount of PVP (g)	Details of secondary excipient if present
5.1.1	6	2	None
5.1.3	6	1.5	0.5 g PAA
5.1.7	6	1.5	0.5 g Sucrose
5.1.9	6	1.5	0.5 g Eudragit S

Table 5.1 The content of each high concentration clotrimazole (75% w/w) dispersion produced in Section 5.2.4.

5.2.5 PRODUCTION AND ANALYSIS OF SPRAY DRIED CLOTRIMAZOLE DISPERSIONS CONTAINING CLOTRIMAZOLE 60% W/W OR 40% W/W

The amount of each component used to prepare the dispersions in this section is stated in Table 5.2, with each dispersion being prepared as follows. Accurately weighed clotrimazole was dissolved in stirred ethanol (100 ml) in a 200 ml conical flask. After 2 h distilled water (30 ml) was then added and the solution was allowed to mix for a further 15 min. This was followed addition of the PVP which was given 15 min to dissolve. Once the PVP had dissolved a secondary excipient was added if required (Table 5.2). The samples were allowed to mix for a further 2 h before being spray dried using the SD Niro spray dryer.

Dispersion number	Amount of Clotrimazole (g)	Amount of PVP (g)	Details of secondary excipient if present
5.2.1	6.0	4.0	None
5.2.2	4.0	6.0	None
5.2.3	6.0	3.0	1.0 g of PAA
5.2.4	4.0	4.5	1.5 g of PAA
5.2.5	6.0	3.0	1.0 g of PHPMA
5.2.6	4.0	4.5	1.5 g of PHPMA
5.2.7	6.0	3.0	1.0 g of sucrose
5.2.8	4.0	4.5	1.5 g of sucrose

Table 5.2 The contents of each spray dried clotrimazole dispersion produced in Section 5.2.5.

The parameters used for spray drying were as follows: The inlet temperature was set to 65 °C and the outlet temperature was set to 45 °C (both ±3 °C). The nitrogen atomisation rate used was 2 kg/h and the drying rate being 20 kg/h. The feed rate was held at 20% (±2%) of the maximum which equated to a feed rate of approximately 3 ml/min. Samples were collected in 100 ml Duran vessels of known weight, before being stored for 48 h under a constant vacuum at room temperature. Following this the samples were weighed and the yield calculated. Samples (800 mg) were transferred to 4 ml Waters vials for storage at the required conditions.

5.2.6 STORAGE CONDITIONS AND ANALYSIS

A desiccator containing approximately 200 g of phosphorous pentoxide (P_2O_5) was prepared to provide 0% RH conditions and was stored at 50 °C. A second desiccator was prepared using 250 ml of a saturated solution (with excess solid) of magnesium chloride ($MgCl_2$) and was stored at 50 °C. At 50 °C the salt produces a RH of 30.5% RH (Nyqvist, 1983).

5.3 RESULTS AND DISCUSSION

5.3.1 SMALL SCALE SOLUBILITY STUDIES

The excipients used in this Section were the same as in previous chapters. The pertinent solubility results from Section 3.2.2 and Section 4.2.1 are summarised in Table 5.3.

Excipient	Solvent used in solubility study, and result		
	Ethanol	**Water**	**Acetone**
PVP	Soluble	Soluble	Insoluble
PHPMA	Poorly Soluble	Soluble	Insoluble
PAA	Soluble	Soluble	Insoluble
Eudragit S	Soluble	Soluble	Soluble
Sucrose	Poorly Soluble	Soluble	Insoluble

Table 5.3 The solubility of excipients in each of the three solvents, where soluble equates to a solubility of at least 10% w/v and insoluble equates to no observable dissolution of the excipient.

Clotrimazole proved to be soluble to at least 100mg/ml in ethanol. It was found to be insoluble in acetone. As was expected no dissolution was observed in water. Flavanone was found to be insoluble in ethanol; however it did prove to be soluble in acetone to at least 100 mg/ml.

5.3.2 THE STABILITY OF FLAVANONE DISPERSIONS STORED AT ROOM TEMPERATURE AND 0% RH

The only secondary excipient that consistently increased the amorphous stability of griseofulvin dispersions was PHPMA. The decision was therefore made to investigate whether this improvement could be detected when another small molecule was used. To this end, flavanone solid dispersions were prepared. The ratio of flavanone to PVP

and PHPMA in these dispersions was kept the same as in the griseofulvin dispersions from the preceding chapter with a drug weight to (total) polymer weight ratio of 4:6. An attempt was made to prepare flavanone dispersions containing the higher ratio of 6:4. However none of the dispersions containing this higher level of flavanone resulted in a completely amorphous material. As was seen with griseofulvin in the previous chapter, it was not possible to use DSC to determine the level of crystallinity. The flavanone showed a propensity to crystallise on heating. Again, as with griseofulvin, the endotherm relating to the evaporation of residual solvent obscured the recrystallisation exotherm.

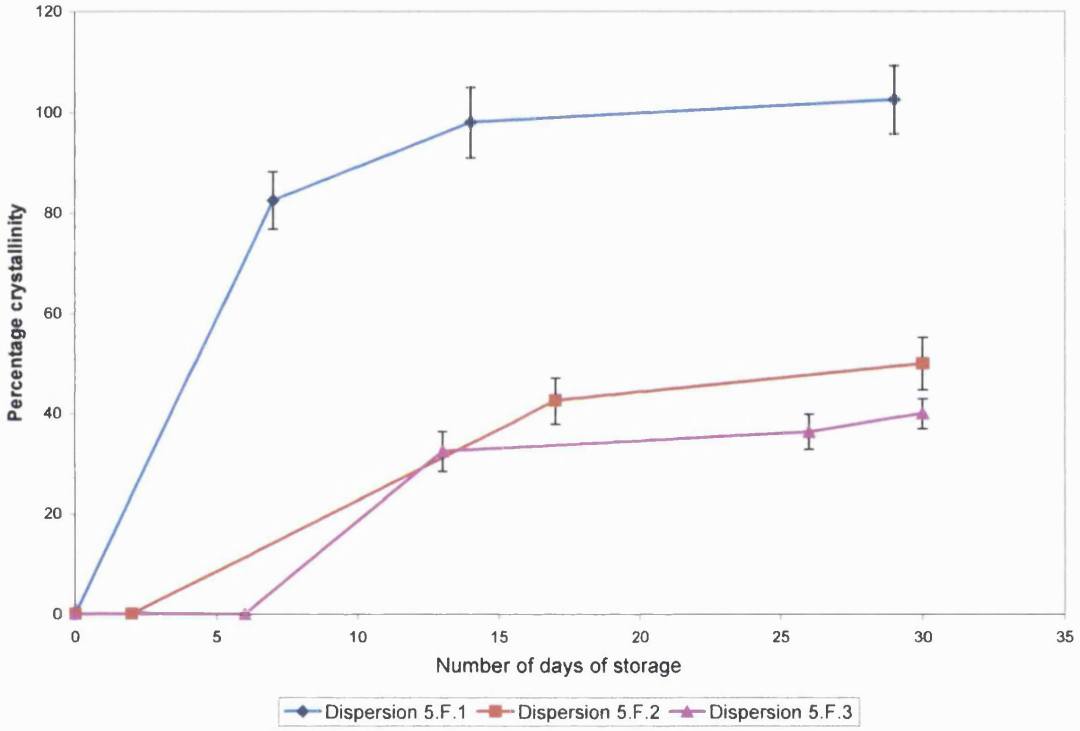

Figure 5.5 The percentage crystallisation of flavanone from solid dispersions of containing varying amounts of PVP and PHPMA over time.

The crystallisation of flavanone from the dispersion when stored at room temperature, 0% RH under vacuum is shown in Figure 5.5. All the dispersions were tested for crystalline material immediately after being spray dried and were found to be completely amorphous. Flavanone crystallised very rapidly in the binary PVP dispersion (Dispersion 5.F.1), and after 7 days this process was nearly complete with 82.5% of the flavanone having recrystallised. After 15 days 98.0% of the flavanone in Dispersion 5.F.1 had crystallised. Dispersion 5.F.2 (15% PHPMA w/w) and Dispersion 5.F.3 (30% PHPMA w/w) were both considerably more stable. After 30 days only

50.0% of the flavanone in Dispersion 5.F.2 and 40.1% in Dispersion 5.F.3 had crystallised (Figure 5.5). Unlike Dispersion 5.F.1 which crystallised very rapidly, both of the tertiary dispersions of flavanone (Dispersion 5.F.2 and Dispersion 5.F.3) had a lag period prior to crystallisation. Overall, this corroborates the observations with griseofulvin dispersions; that the inclusion of PHPMA in the dispersion had a significant effect on stabilising the amorphous form of the drug. This was most probably through an increased level of intermolecular forces, specifically, hydrogen bonding. An attempt was made to locate the T_g for each of the dispersions by DSC (including hyper DSC). Unfortunately the flavanone did not remain amorphous during heating and it proved impossible to locate the T_g. The tertiary dispersions can be seen to reach a plateau phase where the percentage crystallinity begins to level off. This phenomenon was not seen in any of the other dispersions that have been studied, though it was not unexpected. As the drug crystallised phase separation was expected to occur. This should result in the amorphous phase of the dispersion becoming more rich in polymer. This would then inhibit further crystal growth as there would be a consequential increase in the T_g.

5.3.3 The Stability of Dispersions Containing Clotrimazole 75% w/w Stored at Room Temperature and 0% RH

As stated at the beginning of this chapter clotrimazole was selected as a model drug for itraconazole and ketoconazole as it does not contain any proton donating groups but does contain a tertiary amine group which is weakly basic. Therefore it was felt that it would be interesting to see whether the use of a second proton donating polymer would help stabilise the amorphous form.

The results of this stability study showed that there was some merit in the use of tertiary components with systems containing tertiary amines which given the nature of the solvent system from which they had been spray-dried may have been charged. The results are presented in Figure 5.6. Dispersion 5.1.1, which only contained clotrimazole and PVP and the tertiary Dispersion 5.1.9 (Eudragit S) showed the highest levels of crystallisation; 15.6% of the clotrimazole in Dispersion 5.1.1 had crystallised after 60 days whilst 17.5% of the clotrimazole in Dispersion 5.1.9 had crystallised. Dispersion 5.1.3 (PAA) was considerably more stable with only 11.1% of the clotrimazole having crystallised after 60 days. The sucrose dispersion (Dispersion 5.1.7) was the most stable with only 3.5% of the flavanone having crystallised after 60 days.

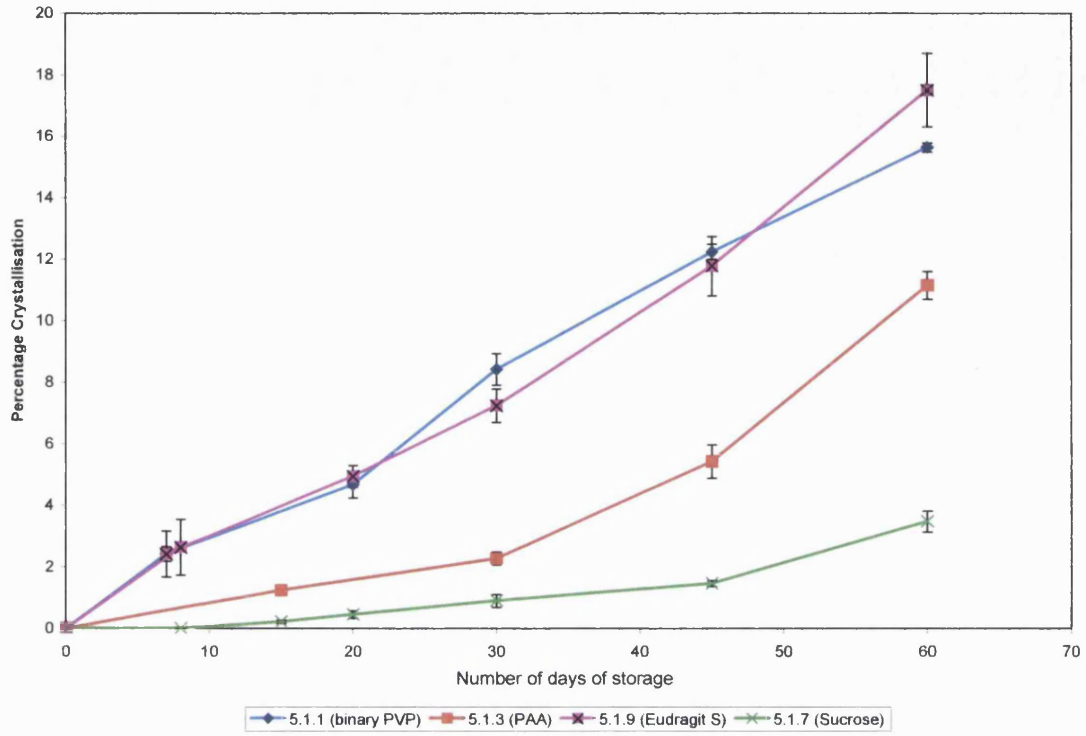

Figure 5.6 The percentage crystallisation of clotrimazole from dispersions that contained clotrimazole 75% w/w, as a function of time.

The T_g of each of the dispersions is presented in Table 5.4. Apart from Dispersion 5.1.7 (sucrose) which had a T_g of 20.3 °C, the dispersions all had a T_g of around 32 °C. Therefore it was not the overall T_g of each of the dispersions that was the dominant factor for the amorphous stability of the clotrimazole dispersions. It should be noted that for these experiments the room temperature was 20 °C ± 3 °C hence the sucrose dispersion was stored on or just below its T_g. This suggests that as with flavanone and griseofulvin, amorphous clotrimazole stability in PVP can be increased by the use of a bridging excipient and that the mechanism by which this occurs is most likely an increase in intermolecular forces.

Dispersion	T_g (°C)
5.1.1 (binary)	32.0 ±1.2
5.1.3 (PAA tertiary)	32.7 ±2.3
5.1.7 (Sucrose tertiary)	20.3 ±1.7
5.1.9 (Eudragit S tertiary)	32.4 ±1.4

Table 5.4 The glass transition temperature of the different dispersions containing 75% clotrimazole w/w.

5.3.4 CLOTRIMAZOLE DISPERSIONS STORED AT 50 °C AND 0% RH

The previous experiments indicated there was merit in the use of a bridging excipient to increase the amorphous stability of clotrimazole in PVP. Clotrimazole dispersions that contained 60% w/w clotrimazole and 40% w/w clotrimazole were prepared and placed on stability study at 50 °C for 13 weeks. This study, unlike the flavanone and griseofulvin studies uses DSC rather than XRPD to investigate the level of clotrimazole crystallisation. Clotrimazole did not display the same propensity to crystallise on heating that both griseofulvin and flavanone did.

DSC allows one to rapidly test samples. The main disadvantage of using DSC to investigate small amounts of crystalline material is that there is a risk of crystallisation being induced on heating. Should this happen, the results would not accurately reflect the amount of crystallisation in the sample prior to the DSC experiment (Section 2.5). In order to counter this 1 in 5 samples, chosen at random was subjected to a Hyper DSC scan at 200 °C/min (Section 2.6). This has been demonstrated to reduce the amount of recrystallisation (Saunders *et al.*, 2004).

The percentage of crystalline clotrimazole detected in spray-dried dispersions stored at 50 °C, 0% RH is shown in Figure 5.7. In all the dispersions containing 60% clotrimazole, crystalline material was seen within the first three weeks. The least stable of these dispersions was Dispersion 5.2.5 containing PHPMA as the tertiary component with 9.5% of the clotrimazole having crystallised after 13 weeks. This was followed by the binary dispersion; Dispersion 5.2.1 in which 6.5% of the clotrimazole crystallised. Both Dispersion 5.2.3 (PAA; 2.4% clotrimazole crystallisation) and Dispersion 5.2.7 (sucrose; 0.6% clotrimazole crystallisation) were considerably more stable.

The 40% clotrimazole dispersions had a similar order of stability. Dispersion 5.2.6 (PHPMA) was the most unstable with 2.6% crystallisation followed by the Dispersion 5.2.2 (binary), with 1.1% crystallisation. Dispersion 5.2.8 (sucrose; 0.3% crystallisation) would appear to been marginally less stable than Dispersion 5.2.6 (PAA; 0.2% crystallisation). The difference in the level of crystalline material between the 2 dispersions after 12 weeks of storage was not significant (Student's *t*-test; $p > 0.05$). That said, during the preceding weeks there was a significant difference between the two dispersions.

One of the primary issues with this set of results was the low level of crystalline material that was seen to develop, which is a concern as in the previous chapter griseofulvin was seen to crystallise on heating. There are a number of pieces of evidence which would support the hypothesis that recrystallisation was not occurring on heating. None of the experiments displayed an observable recrystallisation enthalpy. Solvent loss, which is an endothermic event, could mask recrystallisation as it is an exothermic event. However, in the later weeks the solvent had been effectively removed from the samples by the storage conditions (50 °C, 0% RH). Hyper DSC can be used to inhibit recrystallisation during heating as recrystallisation is an event which is time-dependent (Saunders *et al.*, 2004; Mullin, 2001). The recrystallisation of clotrimazole on heating in the DSC, possibly induced by a high solvent content, would help explain the anomaly of higher levels of crystalline material after 3 weeks seen in Dispersion 5.2.6 (40% clotrimazole with PHPMA) and Dispersion 5.2.8 (40% clotrimazole with sucrose). Yet, in these samples, the results of DSC did not significantly differ when a DSC scan rate of 200 °C/min was used rather than a scan rate of 10 °C/min.

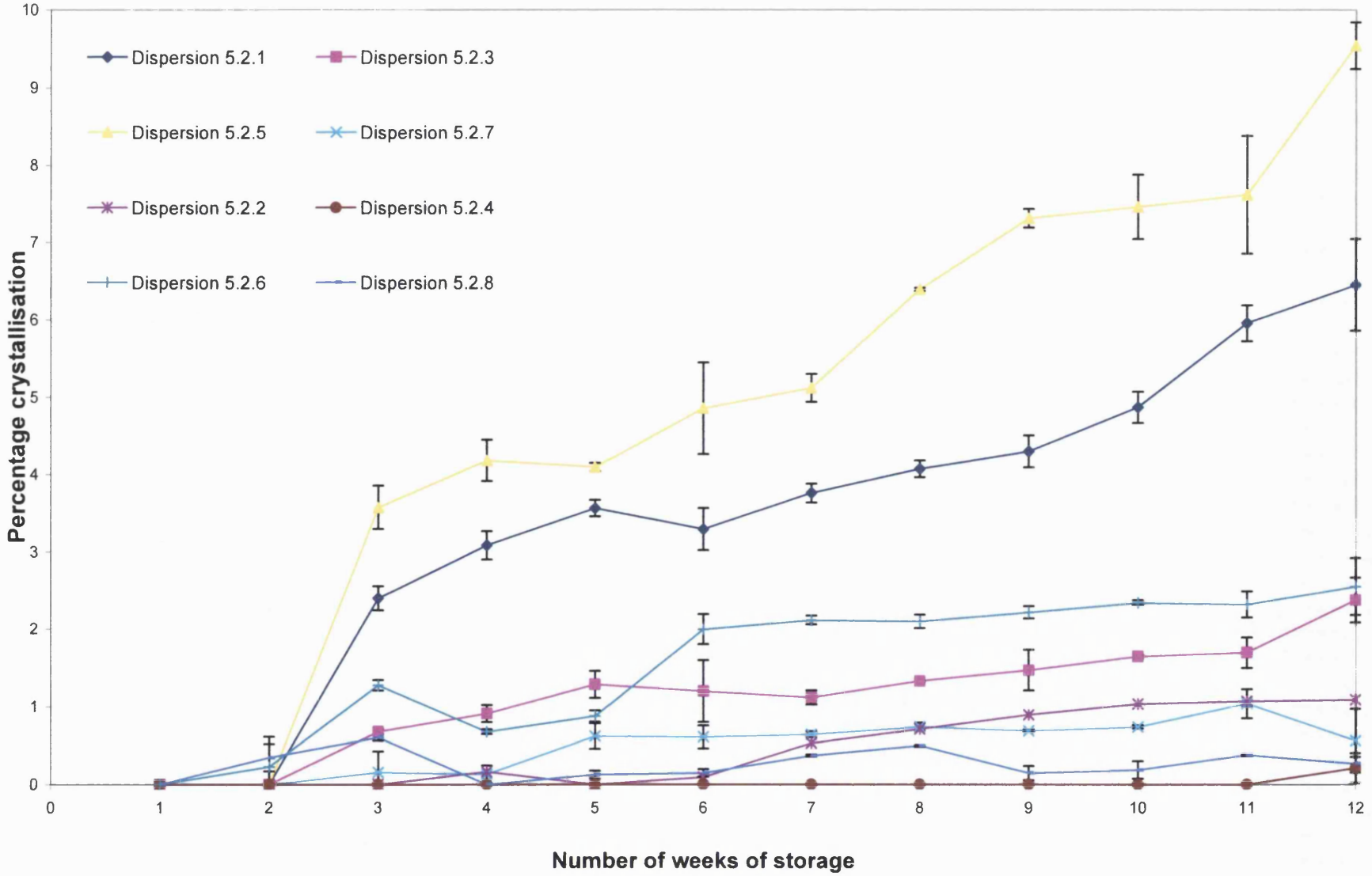

Figure 5.7 The percentage of clotrimazole that crystallised from Spray dried dispersions stored for 12 weeks at 50 °C and 0% RH.

The low level of crystalline material was unexpected. Clotrimazole has a low T_g and as such was expected to crystallise more rapidly than the griseofulvin dispersions examined in Chapter 4. This proved not to be the case and the clotrimazole dispersions are considerably more stable than the griseofulvin dispersions. The high concentration clotrimazole dispersions in which the benefit of a tertiary component appeared to be related to the ratio of hydrogen bond acceptors to molecular mass continues to hold true. In Chapter 4, griseofulvin dispersions containing both PHPMA and PVP were found to be more stable than the binary dispersion containing PVP alone when stored at 50 °C, 0% RH. In the other cases examined (sucrose and PAA), there was either no significant difference in stability or the addition of a secondary excipient proved to be detrimental to amorphous stability. In these clotrimazole dispersions the use of sucrose or PAA resulted in an improvement in amorphous stability. It is possible that the mechanism discussed in Chapter 4 still holds true and that in the case of clotrimazole both PAA and sucrose act as a proton-donating bridge between PVP and clotrimazole. This would not explain the anomaly of PHPMA, the use of which was seen to be detrimental to the amorphous stability of clotrimazole. In Chapter 4 it was observed that PAA and sucrose both increased the rate of crystallisation of griseofulvin, probably by reducing the T_g of the dispersions to a greater extent than they increased stability through direct interactions.

The imadazol amine is a weak base. It is possible that the water-ethanol co-solvent system the dispersions were prepared from was resulting in one of the tertiary amine groups becoming charged thus permitting the clotrimazole to interact directly with PVP. The PVP-amine interaction has been seen in other dispersions such as piroxicam-PVP dispersions, which also displayed signs of hydroxyl interaction with PVP (Tantishaiyakul *et al.*, 1999) and frusemide-PVP dispersions through both a sulphonamide and secondary amide (Doherty and York, 1987). If this were the case PAA may be a particularly effective additive. It can become charged at relatively low pHs and it may be possible to form a complex between clotrimazole and PAA.

The effectiveness of sucrose in stabilising clotrimazole in the dispersions containing clotrimazole 60% w/w was as expected after the early experiments (on dispersions containing clotrimazole 75% w/w) showed that this was the most effective stabiliser. This would lead one to believe that interactions between clotrimazole and the second excipient are important even if the clotrimazole was charged to some degree.

Another possibility suggested by the results from dissolution tests performed on indomethacin dispersions in Chapter 3 is that there could have been two separate phases in the dispersion. This phenomenon was not detected in the DSC and a T_g was observed. It is therefore accepted that this is an unproven hypothesis. In support of the hypothesis was the incomplete dissolution of the dispersion containing Eudragit S in the dissolution media after 8 h even though it had been demonstrated that the indomethacin had completely dissolved after 45 min. (Sections 3.3.5 and 3.3.7). It is noted that PVP and clotrimazole are both soluble in ethanol as is PAA. Sucrose and PHPMA are both poorly soluble in ethanol whilst all the excipients are water soluble (though PHPMA has a tendency to swell instead). It is possible that the solution prior to spray drying was heterogeneous, though the mechanism of addition of solids to the mixing solution prior to spray drying should have limited this risk.

5.3.5 CLOTRIMAZOLE DISPERSIONS STORED AT 50 °C AND 30% RH

Storage at 30% RH caused a marked change in the order in which dispersions appear to be stable (Figure 5.8). In the dispersions containing 60% clotrimazole by weight the stability of amorphous clotrimazole appeared to be related to the T_g of the tertiary excipient used. Dispersion 5.2.7 (sucrose) was by far the least stable and 36.3% of the clotrimazole present crystallised. It was first thought that sucrose might have crystallised, however this was unlikely. The melting point of sucrose is around 180 °C (Yudkin, *et al.,* 1973). When DSC was performed on the dispersion this melt was not detected. In order for sucrose to have crystallised without being detected a sucrose polymorph would have to develop that displayed a T_m of around 140 °C. PVP has been shown to inhibit the crystallisation of sucrose very effectively even under humid conditions (Aso *et al.*, 2002). The XRPD scans of griseofulvin dispersions investigated in Chapter 4 did not show any signs of sucrose crystallisation when stored under similar conditions. There was a slight drop in the onset temperature of the melt (from around 140 °C to approximately 135 °C) but as a decrease in onset temperature was seen in all the dispersions this was ascribed to the presence of the excipients as it has been demonstrated that impurities can lead to a depression of the melting point of a studied substance (Gustin, 1980).

The other dispersions containing clotrimazole 60% w/w all displayed a much lower level of crystallisation than Dispersion 5.2.7 (sucrose). In Dispersion 5.2.3 (PAA) 14.2% of the clotrimazole had crystallised after 13 weeks. 4.3% of the clotrimazole in

Dispersion 4.2.5 (PHPMA) had crystallised, whilst only 2.0% of the clotrimazole in Dispersion 5.2.1 had crystallised over the 13 weeks of storage.

Of the dispersions containing clotrimazole 40 % w/w, 9.7% of the clotrimazole in Dispersion 5.2.6 (containing PHPMA) crystallised and this was the least stable dispersion. Dispersion 5.2.8 was next highest with 6.1% crystallisation. This was followed by Dispersion 5.2.2 which contained 4.2% crystallisation and finally Dispersion 5.2.4 which contained only 1.2% crystallised clotrimazole (Figure 5.8).

Taken in isolation, these results would suggest that the tertiary amine in clotrimazole is charged thus allowing it to interact with PVP and that this charged species could interact with PAA to form a complex. The effect of storing dispersions containing clotrimazole 60% w/w under the same conditions would appear to contradict this as Dispersion 5.2.3 (PAA) was more unstable the Dispersion 5.2.1 (binary) or Dispersion 5.2.5 (PHPMA).

Overall, apart from the sucrose dispersion, the clotrimazole dispersions examined in this section were all very stable. The aim of this section was to improve the stability of amorphous clotrimazole in PVP by adding an extra excipient. However, Dispersion 5.2.1 (binary dispersion) was the second most stable dispersion in terms of the percentage of clotrimazole that crystallised over the 13 weeks.

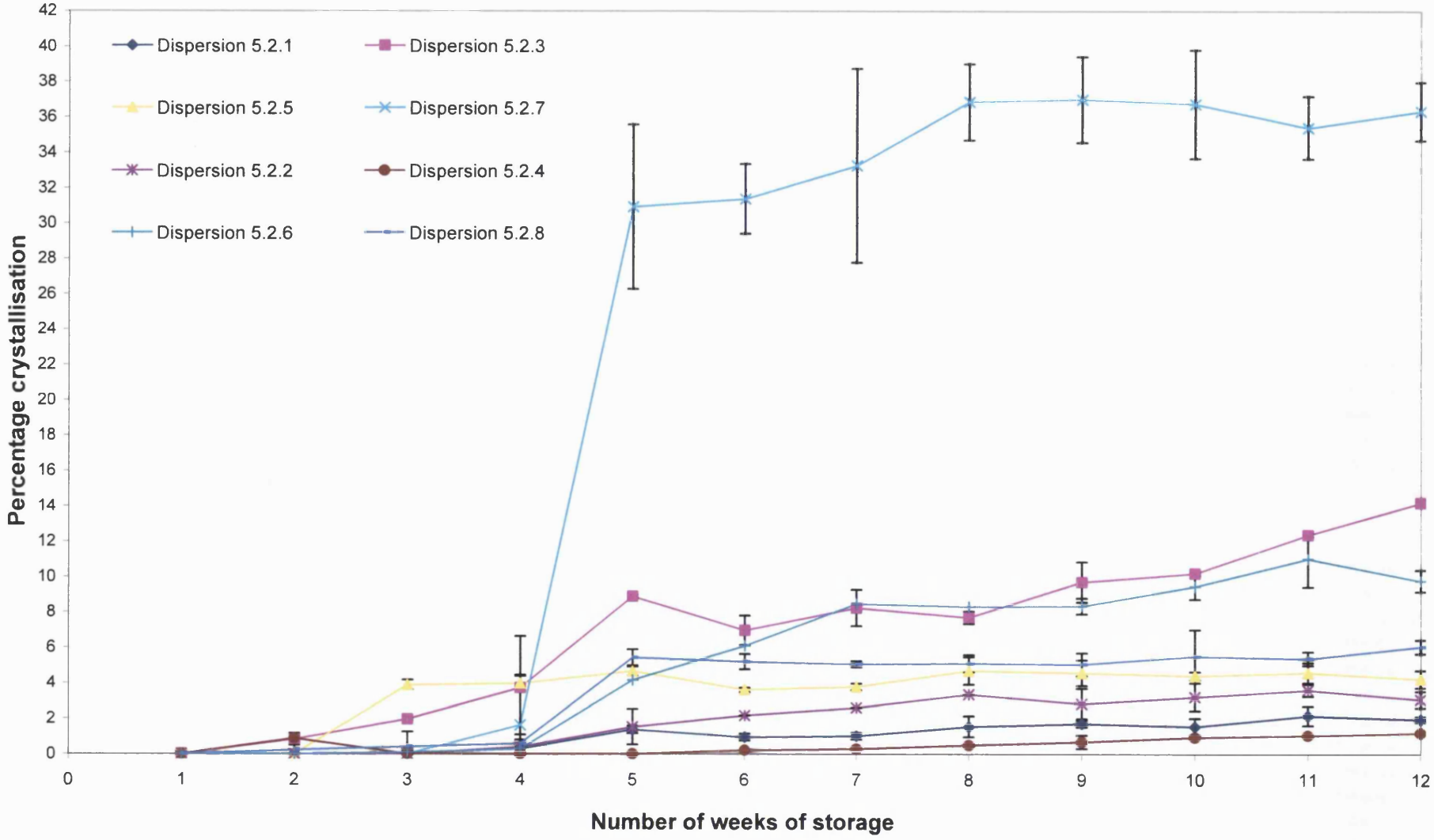

Figure 5.8 The percentage of clotrimazole that crystallised from Spray dried dispersions stored for 12 weeks at 50 °C and 30% RH.

5.4 CONCLUSIONS

There is a clear merit in the use of a second excipient to stabilise a drug in PVP in the amorphous form. This has now been demonstrated in flavanone, PVP and PHPMA dispersions which proved to be more stable than a flavanone and PVP dispersion when stored at room temperature, 0% RH.

The clotrimazole results showed that improvements in amorphous stability were unpredictable, with concentration changes altering the advantage that a secondary excipient might have and on occasion negating any benefit. In the clotrimazole solid dispersions a clear benefit was observed when the clotrimazole made up 75% w/w of the dispersion. This is most likely due to increased levels of hydrogen bonding in the dispersion as the excipient with the lowest T_g provided the greatest benefit. When the dispersions being compared comprised of 40% w/w clotrimazole the dispersion observed to be most stable was that which contained PAA. This proved to be the case at both 0% RH and 30% RH. When clotrimazole made up 60% of the dispersion weight sucrose proved to be the most effective stabiliser when the dispersions were stored at 0% RH. This did not prove to be the case at 30% RH where the binary system proved to be the most stable.

Chapter 6

The Use of Near Infrared Spectroscopy to Characterise the Crystallisation of Griseofulvin Solid Dispersions

6.1 INTRODUCTION

In Chapter 4 the stability of griseofulvin and polyvinylpyrrolidone PVP solid dispersions was investigated using X-ray powder diffraction (XRPD). It was shown that the replacement of some of the PVP with a second excipient could alter the stability. In some cases this was beneficial and in other cases it was detrimental. It was hypothesised that the beneficial effects may be due to the second excipient acting as a proton donating "bridge" between the two proton acceptors; PVP and griseofulvin. XRPD is a time-consuming process. Each sample run lasted 25-30 min and a minimum of three repeats were performed. This meant that to analyse 8 dispersions stored at 5 different conditions took around 60 h to provide 1 data set. There are also other risks associated with XRPD that may result in a large variation in the calculated percentage of crystalline material. These risks were covered in Section 2.7 with Tables 2.2a-c being of particular importance.

Near infrared spectroscopy (NIRS) is a rapid non-invasive technique that requires minimal sample preparation. Sample acquisition is rapid, usually taking around 1-2 min. The dispersions prepared for Chapter 4 (Set One) were used to investigate the possibility of using NIRS as a tool for the assessment of crystallisation from solid dispersions. It was hoped that NIRS would also provide information on the mechanism by which crystallisation was inhibited and the interactions that were occurring between the dispersion constituents. This was considered important as it was originally hypothesised that any second excipient which could act as a proton-donor would be beneficial for stability.

6.2 METHODS

The dispersions used in this chapter are those from Section 4.2.4. The analysis of these dispersions by TGA, DSC and XRPD has already been discussed and SEM images of these dispersions are presented in Appendix One. These dispersions were also analysed by NIRS using spray-dried griseofulvin as one of the reference materials. The method by which this griseofulvin was produced has been presented in Chapter 4, Section 4.2.3.

6.2.1 PREPARATION AND ANALYSIS OF SPRAY DRIED GRISEOFULVIN

Griseofulvin (6 g) was dissolved in stirred acetone (240 ml) in a 500 ml conical flask for a period of 2 h. The solution was then spray dried using the SD Niro as described in Sections 2.3, 4.2.3 and 4.2.4.

Upon spray-drying, griseofulvin (200 mg) was placed in a Waters vial (4 ml). NIRS between the wavelengths 1100-2500nm was then performed on the sample as described in Section 2.9. This was performed in triplicate with the sample being shaken between each spectra being taken. An average spectrum was produced and the standard normal variant (SNV) 2nd-derivative of this spectrum was then compared with an SNV 2nd-derivative of the griseofulvin as supplied in order to identify differences between the two spectra which could be due to the amorphous nature of the spray-dried griseofulvin.

6.2.2 PREPARATION AND ANALYSIS OF GRISEOFULVIN DISPERSION SET ONE

The dispersions presented in this section are those prepared as in Section 4.2.4. Table 6.1 lists their contents for ease of reference

Dispersion number	Amount of Griseofulvin (g)	Amount of PVP (g)	Details of secondary component if present
4.1.1	6.0	4.0	None
4.1.2	4.0	6.0	None
4.1.3	6.0	3.0	1.0 g of PAA
4.1.4	4.0	4.5	1.5 g of PAA
4.1.5	6.0	3.0	1.0 g of PHPMA
4.1.6	4.0	4.5	1.5 g of PHPMA
4.1.7	6.0	3.0	1.0 g of sucrose
4.1.8	4.0	4.5	1.5 g of sucrose

Table 6.1 The contents of each spray dried dispersion studied using NIRS. A repeat of Table 4.1

The storage conditions have already been presented in Chapter 4. Three desiccators containing approximately 200 g of phosphorous pentoxide (P_2O_5) were prepared to provide 0% RH conditions and these were stored at room temperature (RT), 40 °C and 50 °C, whilst two further desiccators were prepared using 250 ml of a saturated solution (with excess solid) of magnesium chloride ($MgCl_2$) and these were stored at room temperature and 50 °C. At room temperature this saturated salt solution produces a relative humidity (RH) of between 32.8% and 33.3%, whilst at 50 °C it produces 30.5% RH (Nyqvist, 1983).

Alongside the TGA, DSC and XRPD analyses that have been discussed in Chapter 4 near infrared (NIR) spectra of the samples were taken on a weekly basis. Each sample was analysed by NIRS with an average of the SNV 2nd-derivative of three spectra being taken immediately upon conclusion of spray drying. The samples were then placed under vacuum for 48 h before being transferred to the pre-prepared desiccators. The samples were then analysed by NIRS every week. At each time point, spectra were taken in triplicate with the vial being shaken in between each sampling. An average spectrum was then produced for each time point. The SNV 2nd-derivative NIR spectra were compared visually to identify changes occurring as a result of crystallisation. The spectra were also analysed using a software package; The Unscrambler® v7.6 which uses Principal Component Analysis (PCA) to calculate which areas of a data set give rise to the largest amount of change in that data set. The data relating to this change were then presented as a scores plot which showed how different the samples were from one another and as a loadings plot which showed where in the data (in this case each spectrum) change is occurring. This is discussed further in Section 6.2.4.

6.2.3 Dynamic Vapour Sorption and Near Infrared Spectroscopy of PVP as Supplied

A sample of PVP (30 mg) was analysed by Dynamic Vapour Sorption and Near Infrared Spectroscopy (DVS-NIRS) as per Section 2.10. The RH cycle used was 0% RH for 300 min (5 h), 30% RH for 240 min (4 h) and finally 0% RH for 240 min (4 h). Whilst in the DVS-NIRS, NIR spectra of the sample were taken every 15 min as described in Section 2.10. PCA was also performed on these spectra.

6.2.4 Principal Component Analysis

Principle component analysis (PCA) is a mathematical method of reorganising information in a data set of samples. Instead of presenting absolute values, new variables, called "Principal Components" (PCs), which account for the majority of change in the data are presented. PCA is a very useful tool for reducing the number of variables in a data set and for obtaining two-dimensional views of a multi-dimensional data set. The data resulting from an NIR scan will consist of 700 variables which will exist in 700-dimensional space as this is the number of dimensions required to plot all of the variables against each other. Whilst it is not possible to visualise this space the concept is the same as that for a two- or three-dimensional space. Figure 6.1 is an

example of the data cloud resulting from a two-dimensional plot of data points across which the first PC (PC 1) has been marked. PC 1 is the direction throughout the data that accounts for the most variability in the data (Davies and Fern, 2004).

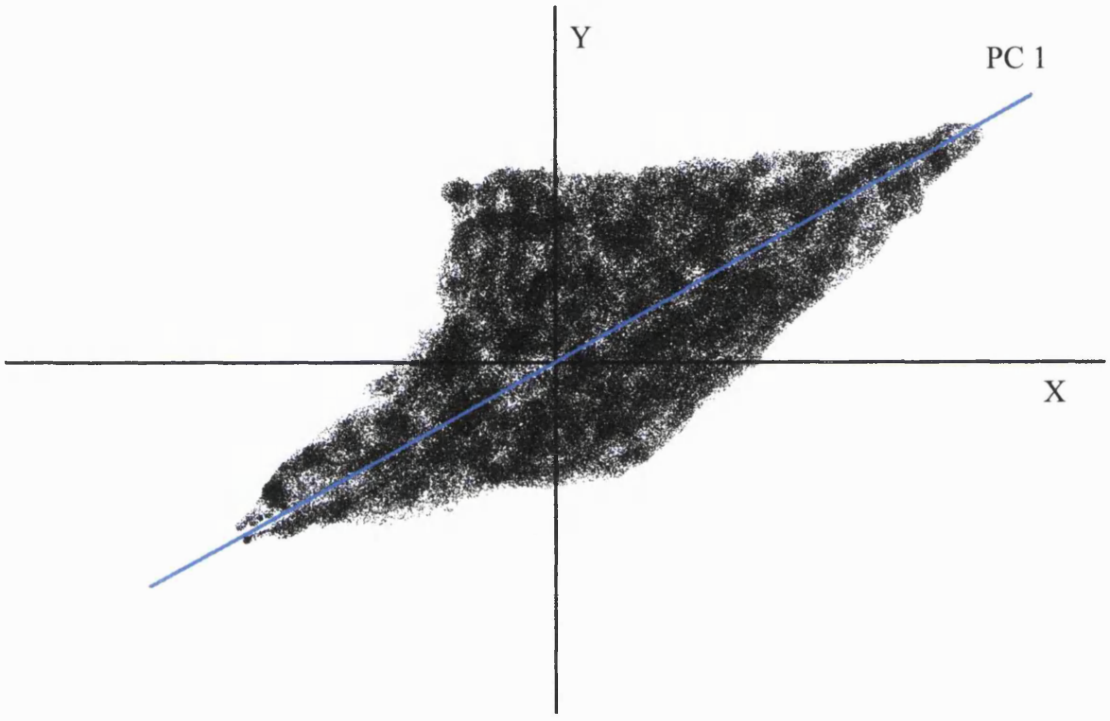

Figure 6.1 The first principal component (PC 1) in a two-dimensional cloud of data points. PC 1 is the direction of the maximum variance.

As stated above, PC 1 covers the largest amount of variability and in turn, subsequent PCs cover the maximum amount of the remaining variability. Therefore in our two-dimension example another line, corresponding the second PC (PC 2) has been indicated (Figure 6.2). As PC 2 is defined to maximise the variability not captured by the preceding factor it has to be orthogonal (at right angles) to PC 1.

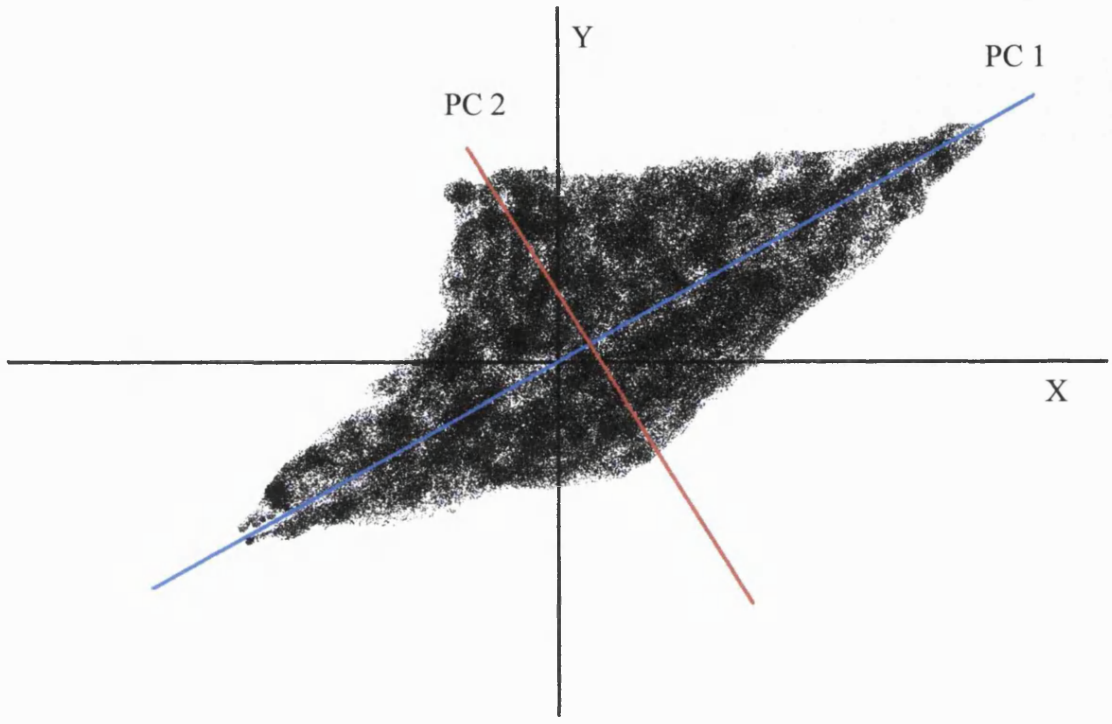

Figure 6.2 The second principle component (PC 2) in a two-dimensional cloud of data points. In a 2-D cloud this covers the remaining variance.

The PCs then form a new set of co-ordinate axes and these replace the original axes. It is important to note that these axes will be dimensionless. With a single variable, the points lie along a line With two variables, they define a point in space and are described by a plane. With three variables, a three-dimensional plot is required, with a plane fitting through the data. With more than three variables, it is not possible to draw or create the plot however the theory remains the same and the axes are rotated to maximise the variance. The first few PCs tend to contain almost all the genuine information whilst later PCs are more likely to describe noise. Therefore one ore two PCs can describe nearly all the variation in a large group of samples with a large number of variables (Jolliffe, 2002; Statsoft, 2003; The Unscrambler®, 2000).

6.2.5 INFORMATION PROVIDED BY PCA

Each PC created during the PCA can be described using three complementary attributes.

- **Variances** shows the proportion of the total variability for which an individual PC is responsible. This is typically expressed as a percentage.

- **Scores** are calculated where information carried by several variables is concentrated onto a few underlying variables. Each sample in the data set has a score along each model PC. The scores show the locations of the samples along each PC and an be used to detect sample patterns, groupings, similarities or differences. A two-dimensional scores plot can be constructed by plotting the value for a particular PC against the score for each sample.

- **Loadings** describe the data structure in terms of variable (wavelength) correlations. Each variable has a loading on a PC. In The Unscrambler® software, a plot of the X-loadings against the X-variables (wavelengths) is given, which looks akin to a NIR spectrum. The wavelengths that contribute the most to a PC will have the largest highest positive or negative loading value, which appears as a peak on maxima or minima on this "spectrum". This may permit the identification of a particular factor that is causing the variation. For example, if a plot looks similar to the NIR spectrum of water with peaks at around 1430 nm and 1950 nm it is likely that the loadings plot results from variations in the water content.

6.3 RESULTS AND DISCUSSION

6.3.1 THE IDENTIFICATION OF AREAS INDICATIVE OF CRYSTALLINE GRISEOFULVIN IN NIR SPECTRA OF THE DISPERSIONS

The SNV 2nd-derivative of the NIR spectra of crystalline griseofulvin and that of amorphous griseofulvin are shown in Figure 6.3. Alongside these is a plot resulting from the subtraction of the amorphous spectrum from the crystalline spectrum. This difference will be referred to as SD-C throughout the text. This plot covers the whole of the NIR spectrum from 1100 nm to 2500 nm. The SD-C plot should help the identification of the spectral changes that occur as a result of crystallisation and that will therefore occur in dispersions on storage.

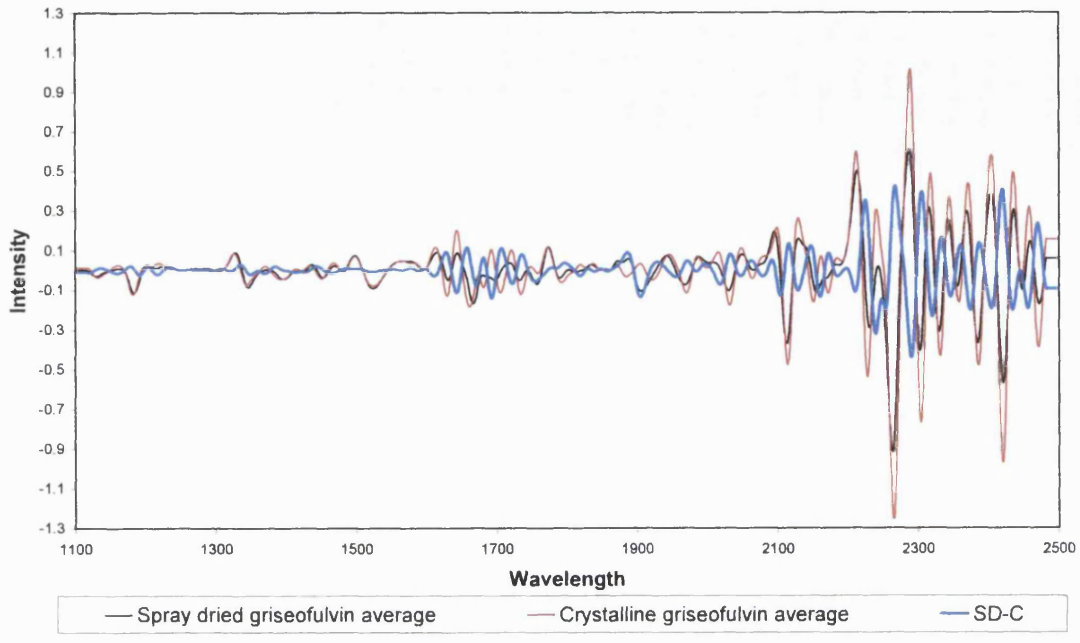

Figure 6.3 Plot of the mean SNV 2nd-derivative of spray dried griseofulvin and the mean SNV 2nd-derivative of crystalline griseofulvin along with a plot of the difference between the two (SD-C).

Figure 6.4 shows the spectra of all the excipients alongside the SD-C plot. This was used to ascertain which of the changes in the griseofulvin spectra were least likely to be obscured by random variation in excipient peaks.

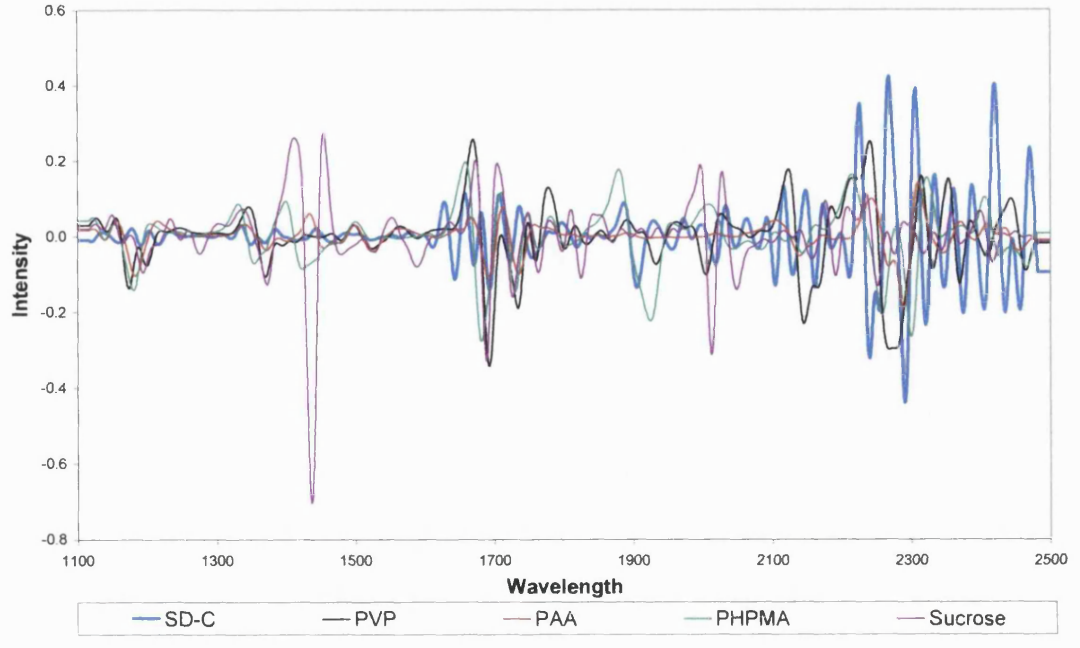

Figure 6.4 Plot of the mean SNV 2nd-derivative of all the excipients used to produce dispersions along with a plot of SD-C.

Figures 6.5a-6.5c cover three regions of the NIR spectra where differences between the two spectra can be seen. Alongside a plot of the difference between the two griseofulvin spectra are spectra relating to all the excipients found in the griseofulvin dispersions. It is important to note at this juncture that the spectrum for sucrose is that of the crystalline material. The diagnostic peak can be either a positive or negative peak as it is merely a difference that is required.

Figure 6.5a shows the range 1600-1700 nm which contains a number of small diagnostic peaks. Peaks were found at 1628 nm and 1644 nm and these showed very little interference from the excipient peaks. Peaks were found at 1658 nm and 1682 nm though these showed some interference from the peaks in the spectrum for PHPMA. There were also peaks at 1670 nm and 1694, however these were expected to be obscured by peaks in both the PVP and crystalline (and possibly amorphous) sucrose spectra.

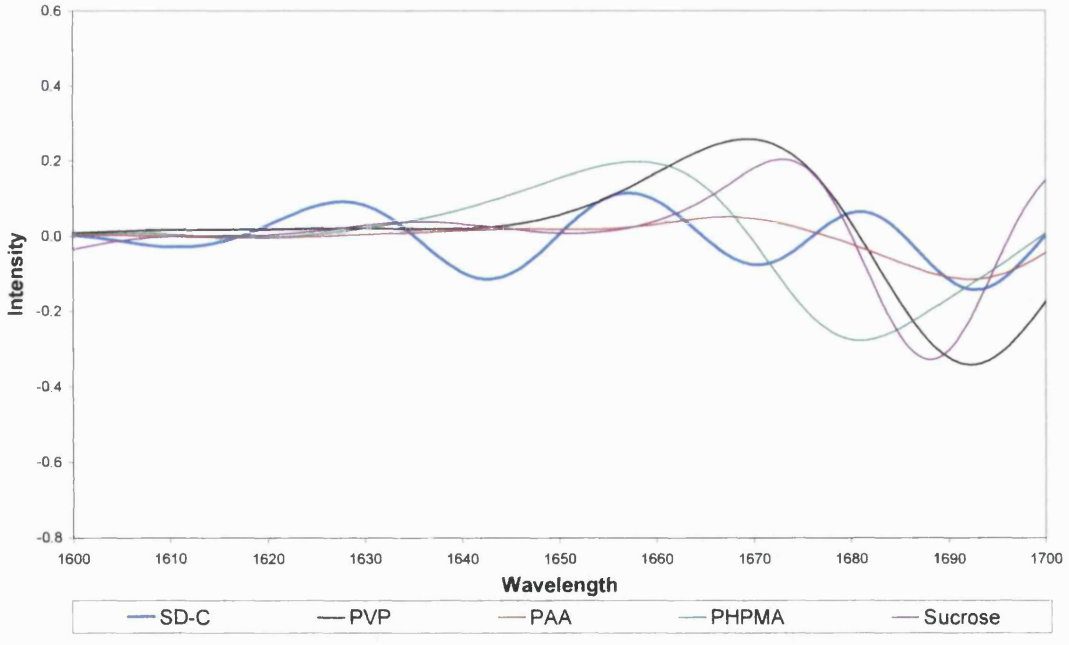

Figure 6.5a Plot of the mean SNV 2nd-derivative of all the excipients used to produce dispersions along with a plot of SD-C covering 1600-1700 nm.

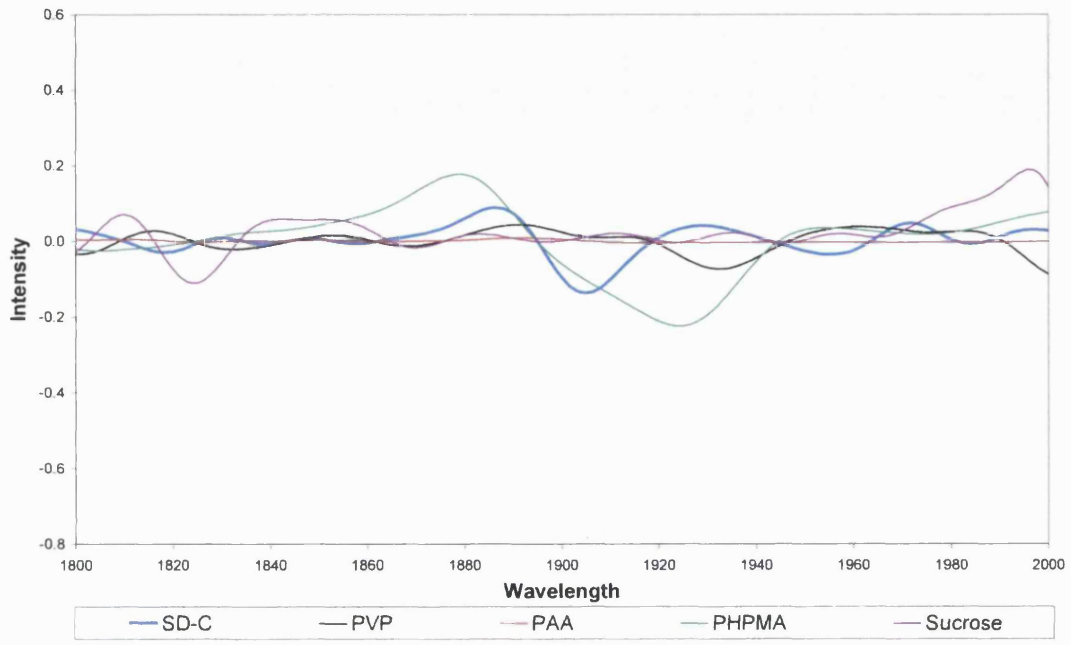

Figure 6.5b Plot of the mean SNV 2nd-derivative of all the excipients used to produce dispersions along with a plot of SD-C covering 1800-2000 nm.

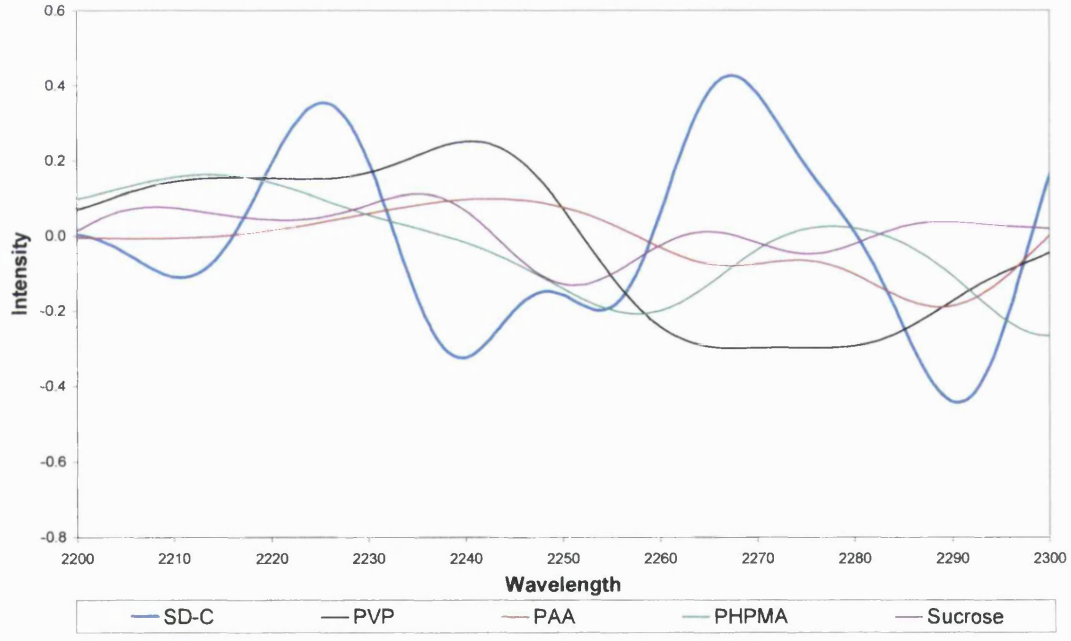

Figure 6.5c Plot of the mean SNV 2nd-derivative of all the excipients used to produce dispersions along with a plot of SD-C covering 2200-2300 nm.

In Figure 6.5b (1800-2000 nm) only one peak free from interference at 1906 nm was considered to be of interest. One of the major problems with this area was that water peaks would undoubtedly be present and these would cause interference from around 1900 nm and upwards (Seisler *et al.*, 2002), therefore it was considered best to avoid using this region as changes in water content could be mistaken for crystallisation. Also

by separating the changes in the water region from the changes in crystallisation an investigation could be made into whether changes in the two correlated.

Figure 6.5c shows the region 2200-2300 nm. This region displayed the largest peaks. A large double peak was found between 2230 and 2260 nm which though it was expected to suffer from some interference was considered to be very useful and it was found between two positive peaks at 2226 nm and 2268 nm. At 2290 nm there was another large peak which was relatively free from interference. It was therefore decided that the investigations into the crystallisation of griseofulvin from the solid dispersions would concentrate on this area.

6.3.2 The Crystallisation of Griseofulvin from Dispersions Containing 60% Griseofulvin w/w Stored at 0% Relative Humidity

Having identified peaks within certain regions of the NIR spectrum as being indicative of crystallisation the next step was to take one of these regions and see if on storage the dispersions showed a similar change. The 4 dispersions containing griseofulvin 60% w/w (Dispersion 4.1.1, Dispersion 4.1.3, Dispersion 4.1.5 and Dispersion 4.1.7) were analysed as a group at each of the different temperatures. PCA was used to prepare scores and loading plots of the changes occurring in the dispersions.

As was explained in more detail in Section 6.2.4, the loading plots show the wavelengths of the spectra which result in the largest amount of change in the sample set. If griseofulvin had crystallised than one would expect the loading plot of the wavelengths 2200-2300 nm to look like that of the SD-C plot shown in Figure 6.5c. Figure 6.6 shows the largest principal component (PC 1) located between 2200-2300 nm for those samples stored at room temperature, 0% RH. The double peak between 2230 and 2260 nm was seen in both the SD-C plot and the plot in Figure 6.6. PC 1 in this case makes up 88% of the variance between the spectra. This provides a rational means to identify crystallisation in the solid dispersions.

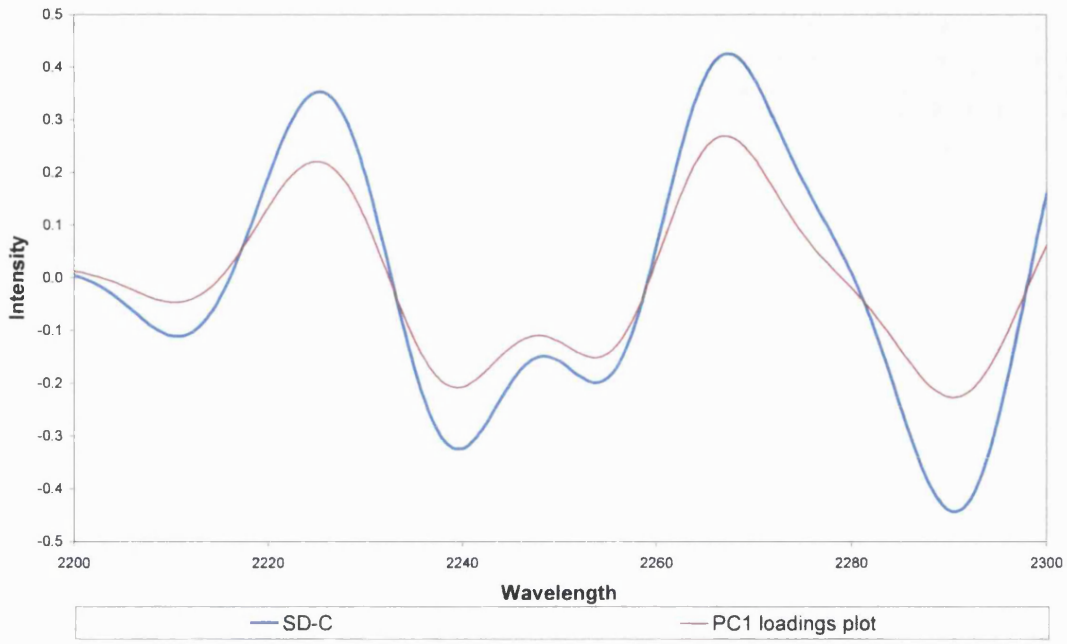

Figure 6.6 The PC 1 loadings plot for all of the dispersion samples containing 60% griseofulvin by weight stored at room temperature and 0% RH compared with the SD-C plot.

The scores plot describes where the changes in loadings between individual spectra are occurring. As crystallisation will only proceed in one direction, i.e. griseofulvin will not "un-crystallise" a smooth slope should be detected. Figure 6.7 shows the scores plot related to the loadings plot in Figure 6.6. Apart from Dispersion 4.1.5 (PHPMA) a consistent decrease was seen in the section of the scores plot pertaining to each of the individual dispersions. The values for the section of the scores plot relating Dispersion 4.1.5 remained relatively constant. From this it was concluded that Dispersion 4.1.5 was more stable then the other dispersions and that PHPMA was therefore a more effective stabiliser of the amorphous form. The consistent decrease seen with the other dispersions suggested that the changes in the NIR spectra related to the crystallisation of griseofulvin was similar for all these dispersions. This would suggest that on a molecular level the mechanism by which griseofulvin crystallised was the same.

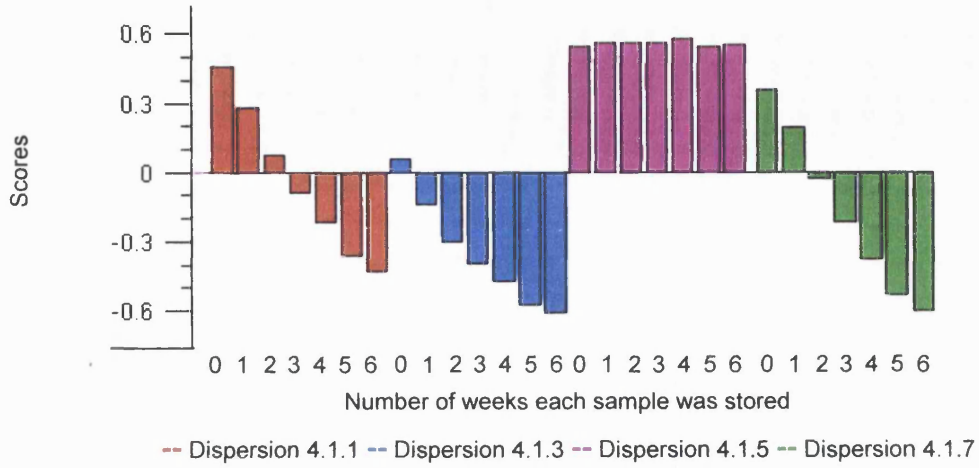

Figure 6.7 The PC 1 scores plot for all of the dispersion samples containing 60% griseofulvin by weight stored at room temperature and 0% RH.

Figures 6.8 and 6.9 show the loading plots for the storage of dispersions at 40 °C and 50 °C respectively. Overlaid onto each of these is the SD-C plot for griseofulvin. The loadings plots and the SD-C plot proved to be near identical. This showed that crystallisation had again occurred in these two samples.

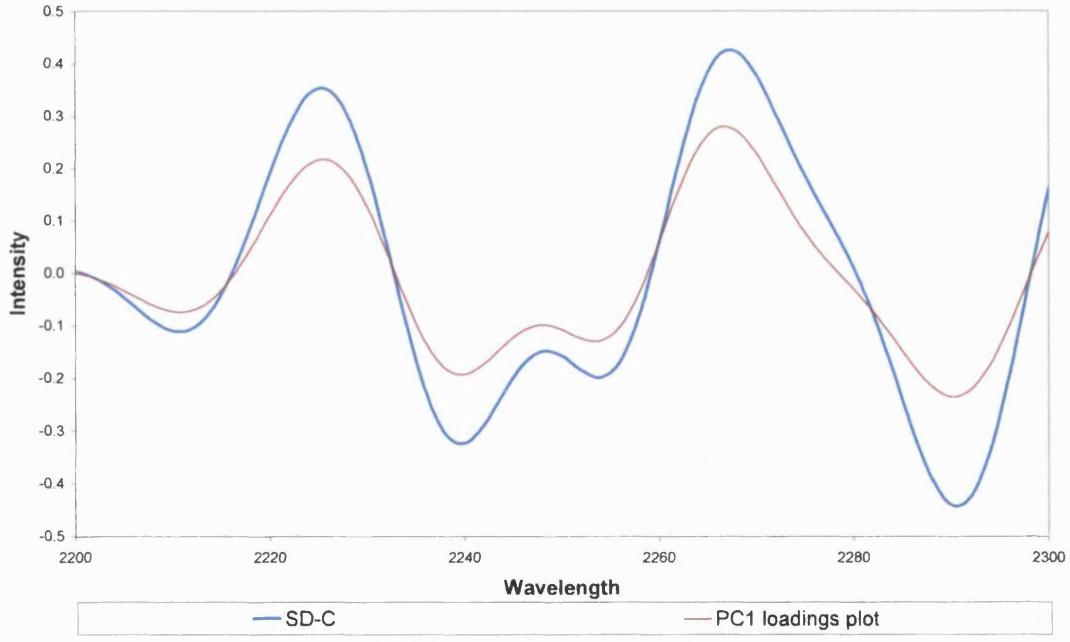

Figure 6.8 The PC 1 loadings plot for all of the dispersion samples containing 60% griseofulvin by weight stored at 40 °C and 0% RH compared with the SD-C plot.

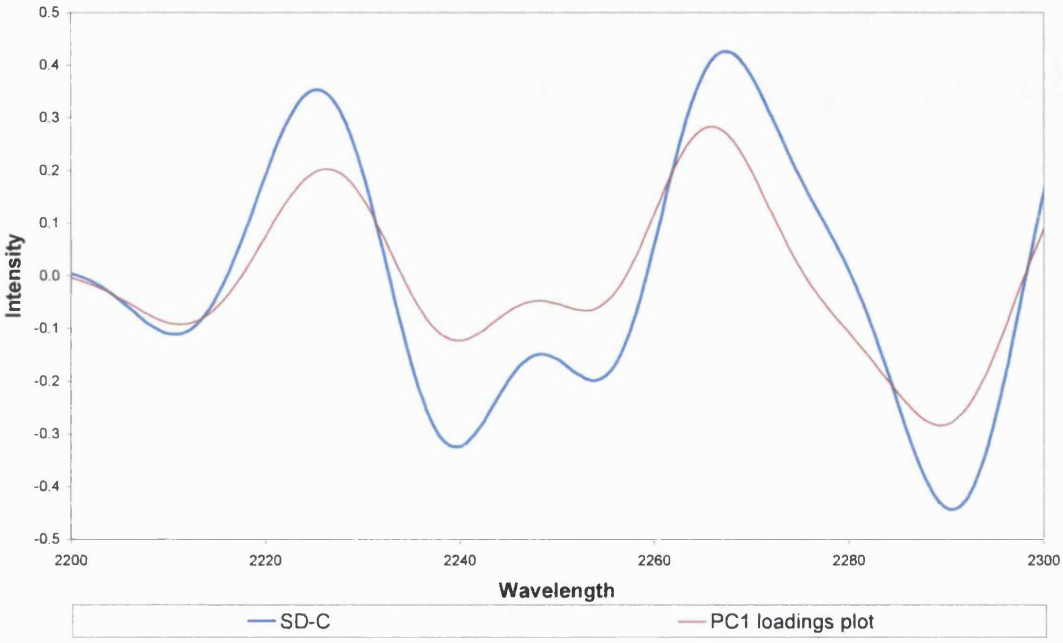

Figure 6.9 The PC 1 loadings plot for all of the dispersion samples containing 60% griseofulvin by weight stored at 50 °C and 0% RH compared with the SD-C plot.

Figure 6.10 shows the scores plot for the samples stored at 40 °C. As with the samples stored at room temperature, amorphous griseofulvin in Dispersion 4.1.5 (PHPMA) proved to be the most stable. Dispersion 4.1.3 (PAA) and Dispersion 4.1.7 (sucrose) showed the largest change between weeks 0 and 1 whilst Dispersion 4.1.1 (binary) showed the largest change between weeks 1 and 2. After this initial large change the scores plot values were relatively consistent and no real trend was observed. Of the 4 dispersions, Dispersion 4.1.7 would appear to have been the least stable as the section of the scores plot covering Dispersion 4.1.7 had the largest change overall. This suggested that the majority of the crystallisation occurred within the first two of weeks of storage and that in the weeks that followed there was no real change.

In Figure 6.11 (which is a reproduction of some of the data in Figure 4.9), the results of the XRPD experiments on the dispersions are presented. They demonstrate that whilst a considerable amount of crystallisation had taken place during the first three weeks of storage in all the dispersions far more crystallisation took place in the ten weeks that followed. One would therefore expect to a more linear scores plot like that observed for the PHPMA dispersion (Dispersion 4.1.5). One possibility is that the NIR data was detecting crystal nucleation and not crystal growth.

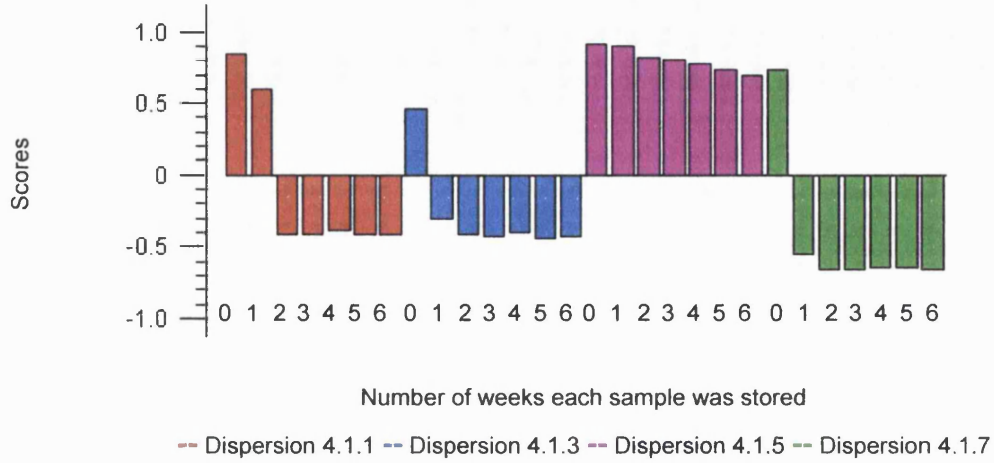

Figure 6.10 The PC 1 scores plot for all of the dispersion samples containing 60% griseofulvin by weight stored at 40 °C and 0% RH.

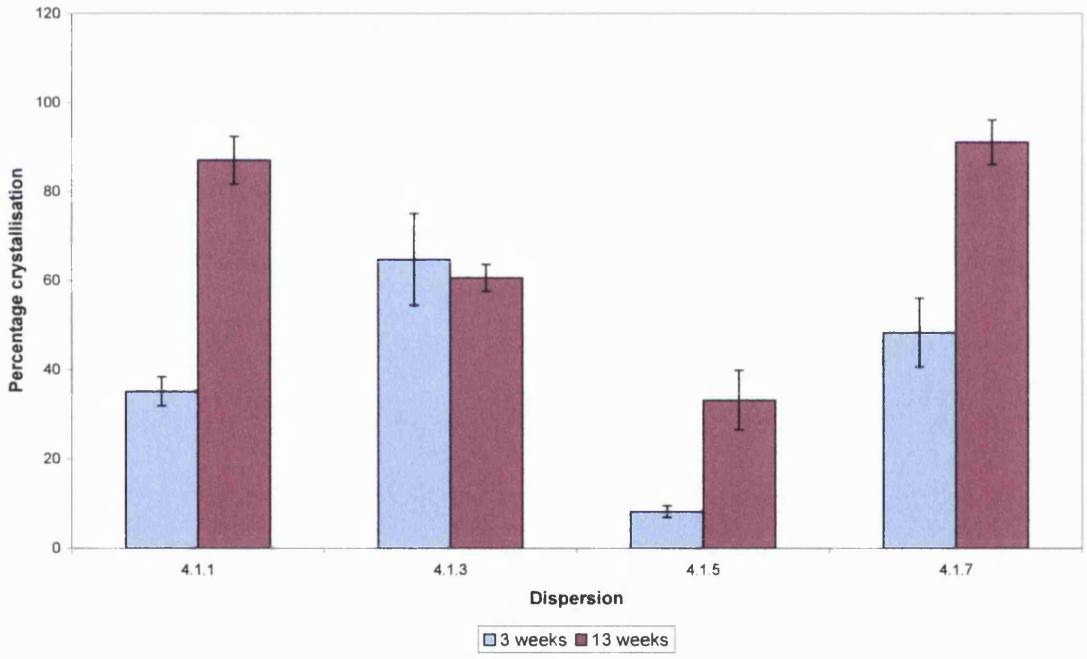

Figure 6.11 The crystallisation of griseofulvin dispersions containing 60% griseofulvin by weight stored at 40 °C and 0% RH for 13 weeks when studied by XRPD. This is a reproduction of some of the data in Figure 4.9.

As was seen when the dispersions were stored at 40 °C, at 50 °C there was a similar disparity between the data that was produced from PCA of the NIR spectra and the data that was generated by XRPD. The sections of the scores plot (Figure 6.12) covering Dispersion 4.1.1 (binary), Dispersion 4.1.3 (PAA) and Dispersion 4.1.7 (sucrose) showed that the largest change that occurred in the dispersions was between week 0 and

week 1. The scores plot for Dispersion 4.1.5 (PHPMA) would suggest a more constant rate of crystallisation. This is not what would be expected when the XRPD data was re-examined (Figure 6.13). The XRPD data seemed to suggest that in later weeks there was as much crystallisation as in early weeks.

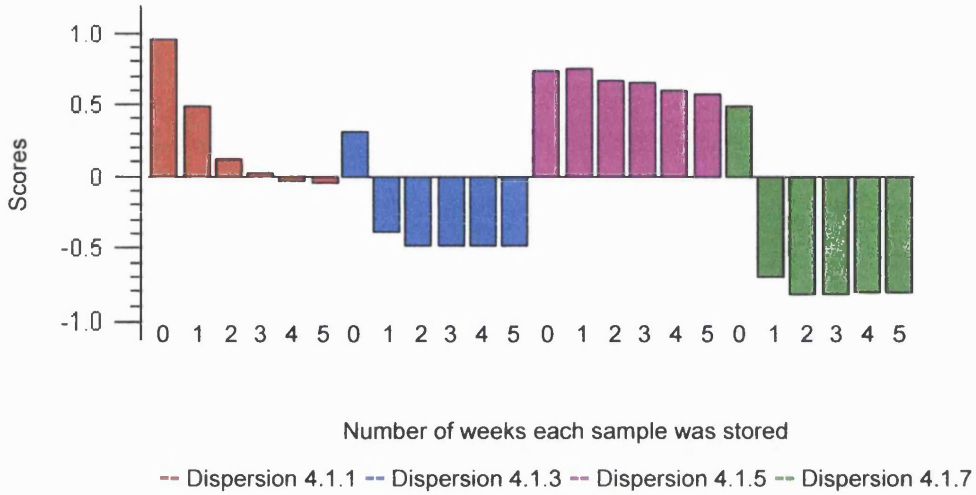

Figure 6.12 The PC 1 scores plot for all of the dispersion samples containing 60% griseofulvin by weight stored at 50 °C and 0% RH.

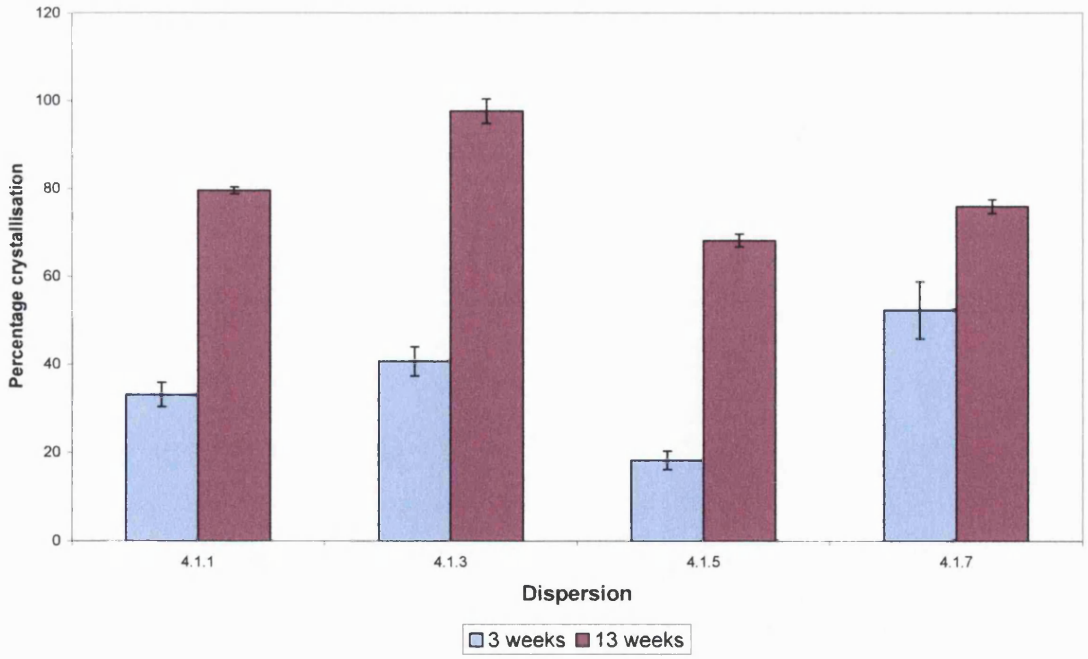

Figure 6.13 The crystallisation of griseofulvin dispersions stored at 50 °C 0% RH for 13 weeks. This is a reproduction of some of the data in Figure 4.9.

It may be that NIRS can in some way "predict" crystallisation where XRPD can only detect it. XRPD detects the long range order that is considered to be true crystallinity. If the NIR scans were detecting short range order which could be occurring quite

rapidly in the initial weeks and then followed by either crystal growth or reorientation of the pockets of order in the still amorphous system. It is possible that the NIR is only sensitive to a narrow band of change and that should a critical concentration of crystalline material be reached the NIR no longer detects increases in the level of crystalline material. However, whilst the amorphous griseofulvin in Dispersion 4.1.5 (PHPMA) was demonstrated to be more stable it did crystallise from the dispersion to a considerable degree over the 13 weeks of storage. One would therefore expect the shape of the portion of the scores plot covering the spectra for Dispersion 4.1.5 to differ from the shape of the scores plots for the other dispersions. One would also expect that the start and end point of the scores plot would be the same for Dispersion 4.1.5 as for the other dispersions. This did not prove to be the case.

Thus far only the largest principal component, PC 1 has been discussed. For the dispersions stored at room temperature this made up 88% of the variance between the sample spectra. The scores and loading plots for PC 2 which makes up 9% of the variance occurring in the different data sets are shown below in Figures 6.14 and 6.15.

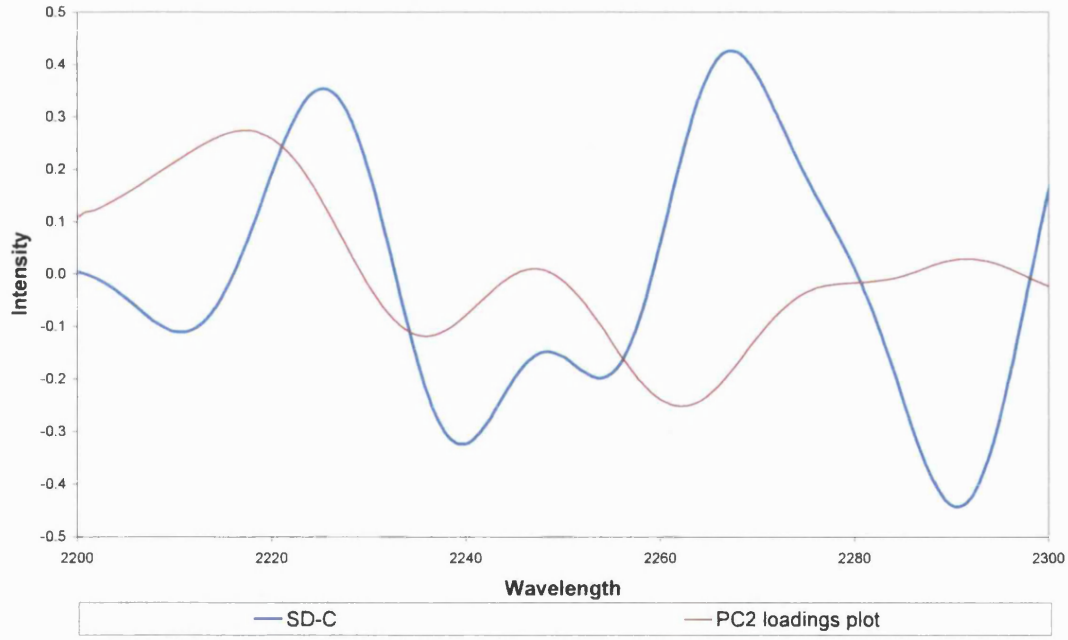

Figure 6.14 The PC 2 loadings plot for all of the dispersion samples containing 60% griseofulvin by weight stored at room temperature and 0% RH compared with the SD-C plot.

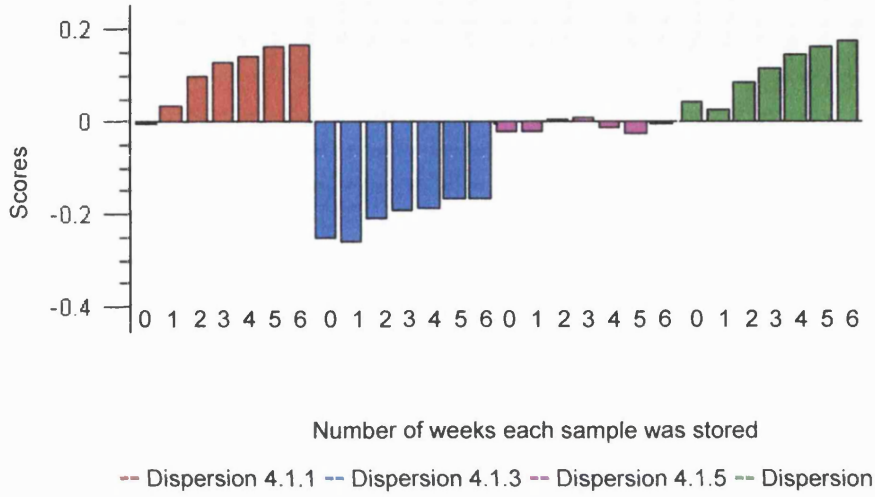

Figure 6.15 The PC 2 scores plot for all of the dispersion samples containing 60% griseofulvin by weight stored at room temperature and 0% RH.

There did appear to be a slight upward shift in the scores for Dispersion 4.1.1 (binary), Dispersion 4.1.3 (PAA) and Dispersion 4.1.7 (sucrose) whilst Dispersion 4.1.5 (PHPMA) remained relatively constant. This suggested that PC 2 may be of some relevance. Unfortunately the scores plots for PC 2 for the dispersion samples stored at 40 °C and 50 °C (Figure 6.16 and Figure 6.17) did not display any trends. In these two cases the PC 2 only related to 6% of the variance observed between the samples which was considerably less than the 9% of the variance that PC 2 made up for dispersions stored at room temperature. Furthermore, when the loadings plots for all three sets of conditions were overlaid (Figure 6.18) there was no consistency in the changes seen in the spectra of the different data sets. This suggested that PC 2 was most probably noise in all cases and this might have been due to slight peak shifts around the areas of the spectra which resulted in the PC 1 crystallisation peaks.

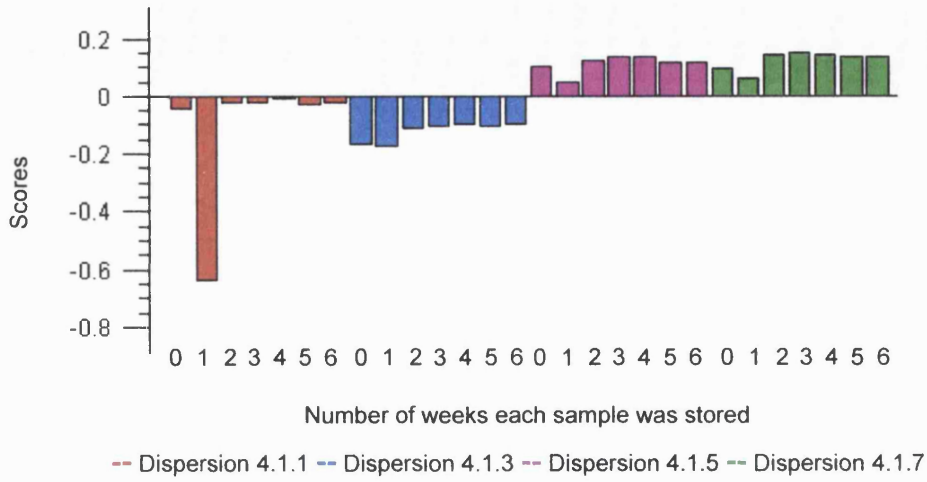

Figure 6.16 The PC 2 scores plot for all of the dispersion samples containing 60% griseofulvin by weight stored at 40 °C and 0% RH.

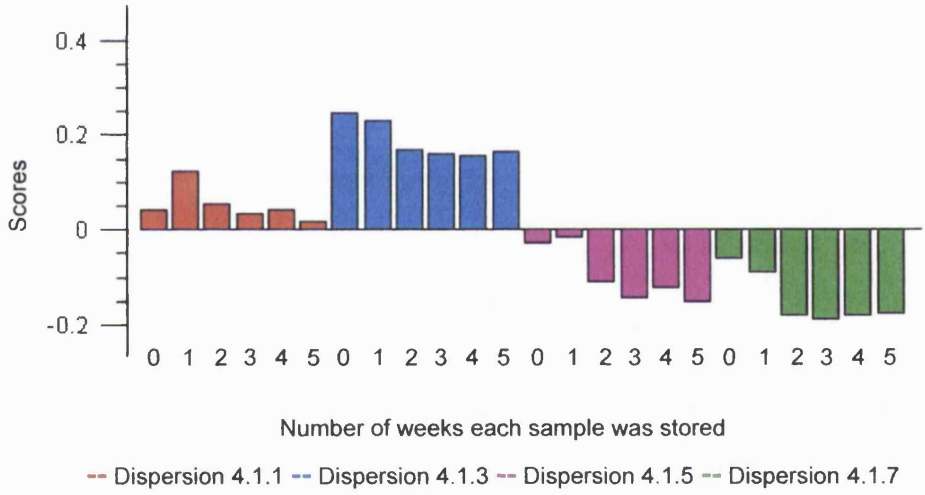

Figure 6.17 The PC 2 scores plot for all of the dispersion samples containing 60% griseofulvin by weight stored at 50 °C and 0% RH.

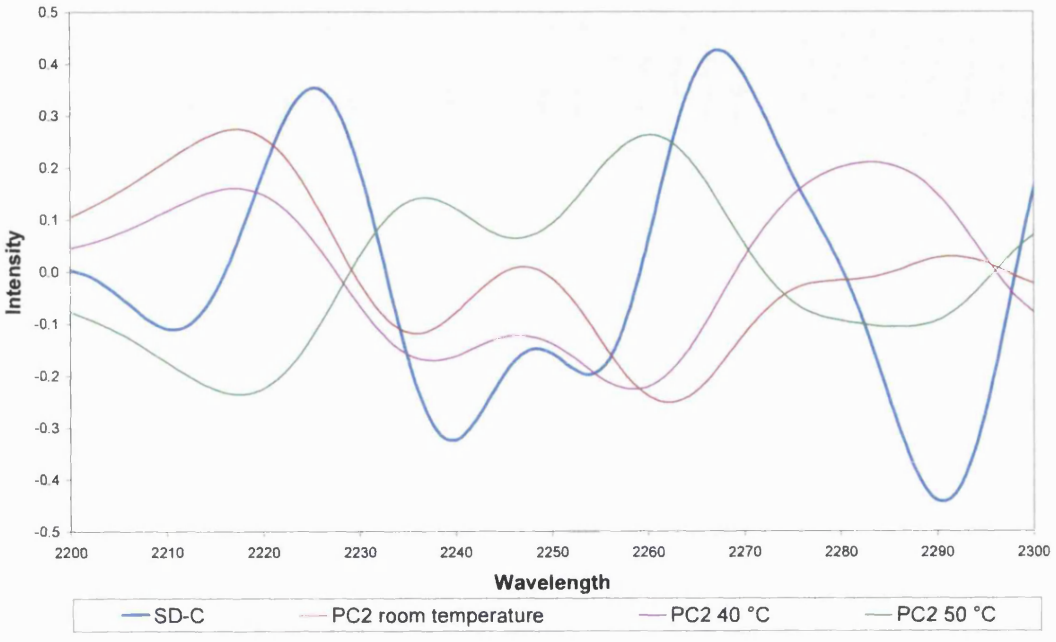

Figure 6.18 The PC 2 loadings plot for all of the dispersion samples containing 60% griseofulvin by weight stored at 0% RH separated by temperature of storage.

6.3.3 The Crystallisation of Griseofulvin from Dispersions Containing 60% Griseofulvin w/w Stored at 30% Relative Humidity

The scores plot and the loadings plot for the dispersions stored at room temperature and 30% RH are shown in Figure 6.19 and Figure 6.20. Apart from one probable outlier that was seen after 2 weeks of storage in the section of the score plot related to Dispersion 4.1.3 (PAA) there was a general consistency between Dispersion 4.1.1 (binary), Dispersion 4.1.3 (PAA) and Dispersion 4.1.7 (sucrose) in that a sudden change occurred during week 1. This was followed by a more gradual change. Dispersion 4.1.5 (PHPMA) differed and the values for the scores plot remained relatively consistent throughout the weeks of study. PC 1 made up 88% of the variance detected in these dispersions and as can be seen from the loading plot (Figure 6.20) the change observed was the same as that detected in the previous dispersions.

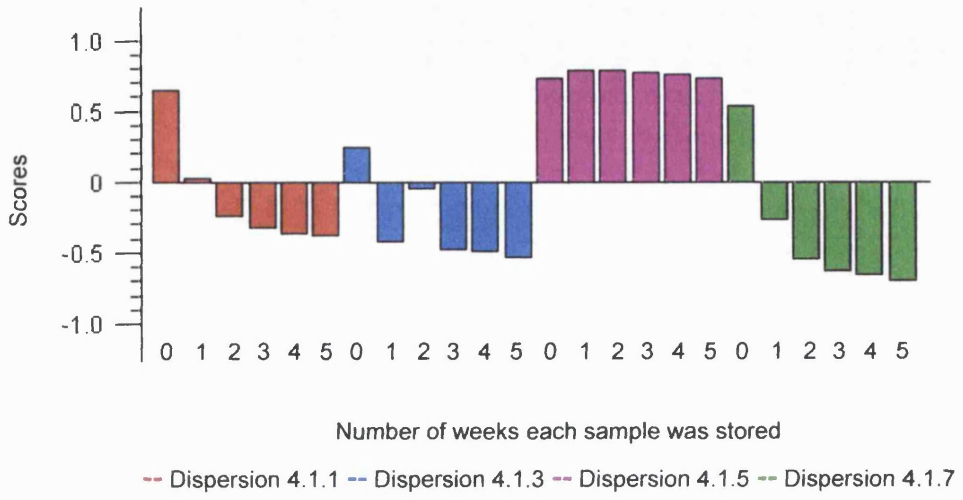

Figure 6.19 The PC 1 scores plot for all of the dispersion samples containing 60% griseofulvin by weight stored at room temperature and 30% RH.

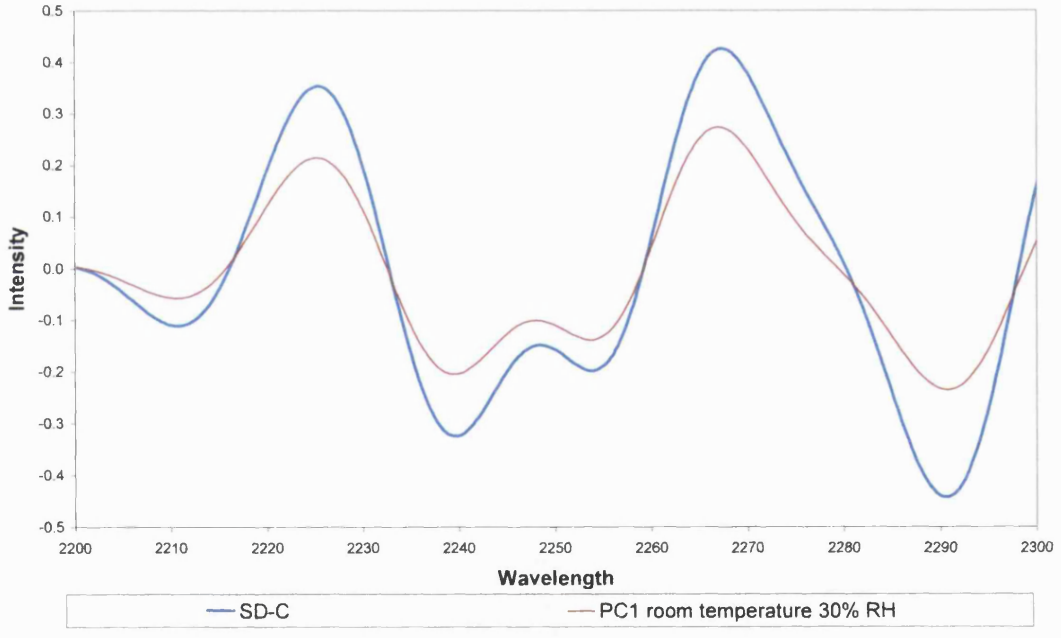

Figure 6.20 The PC 1 loadings plot for all of the dispersion samples containing 60% w/w griseofulvin stored at room temperature and 30% RH compared with the SD-C plot.

The PC 1 loadings plot for all the samples of the dispersions containing 60% w/w griseofulvin stored at 50 °C and 30% RH is shown in Figure 6.21. PC 1 was again the same shape as the SD-C plot. The scores plot was particularly interesting as Dispersion 4.1.3 (PAA) would appear to be have been more stable than any of the other dispersions

including Dispersion 4.1.5 (PHPMA) (Figure 6.22). Whilst the other dispersions underwent the largest change after the first week of storage it was only after 3 weeks of storage that a large shift occurred in the scores plot for Dispersion 4.1.3 (PAA). The XRPD results presented in Chapter 4 (shown in Figure 6.23 for convenience) contradicted the results of the PCA presented in the scores plot. After 3 weeks 81.7% of the griseofulvin in Dispersion 4.1.3 (PAA) had crystallised, and this was the least stable dispersion by some distance, with the next most unstable dispersion, Dispersion 4.1.7 (sucrose) having about half as much crystallisation. In Dispersion 4.1.1 (binary) 32.7% of the griseofulvin had crystallised. Only 26.6% of the griseofulvin in Dispersion 4.1.5 (PHPMA) had crystallised and in Dispersion 4.1.7 (sucrose) 43.1% had crystallised. After 13 weeks of storage all the griseofulvin in each of the dispersions had crystallised. All this leads to the conclusion that NIR spectroscopy is detecting a different change that whilst linked to the change detected by XRPD would appear to be very different.

The PC 2 scores and loadings plots have not been presented here but as with the samples stored at 0% RH there were no obvious trend in the scores plots and the loadings plots did not overlay each other. They did not appear to be similar to any of the other sample loading plots.

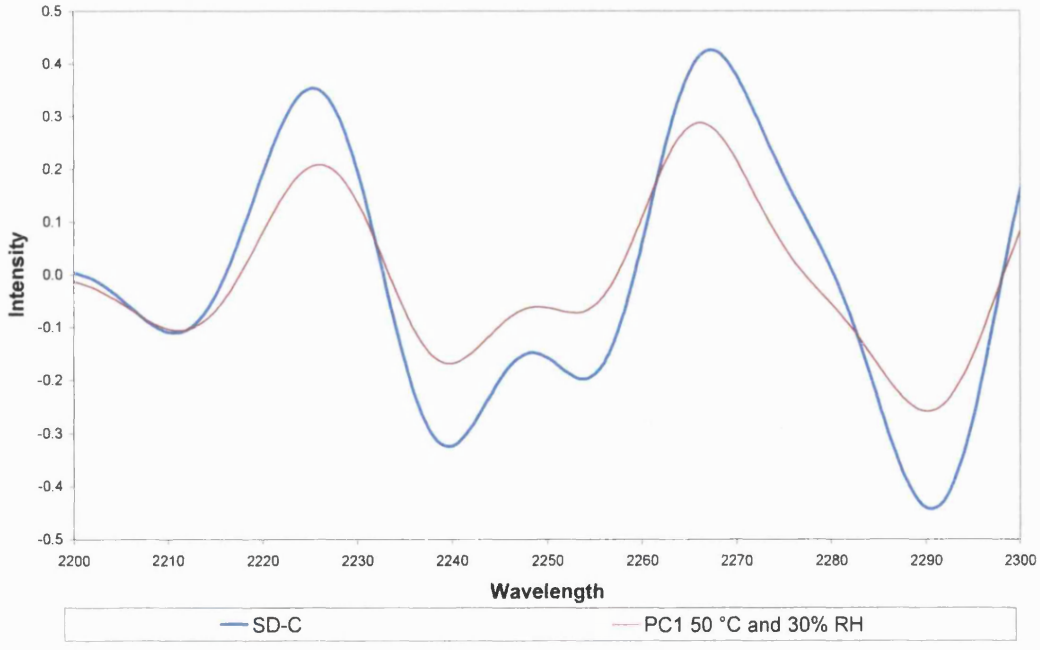

Figure 6.21 The PC 1 loadings plot for all of the dispersion samples containing 60% griseofulvin by weight stored at 50 °C and 30% RH.

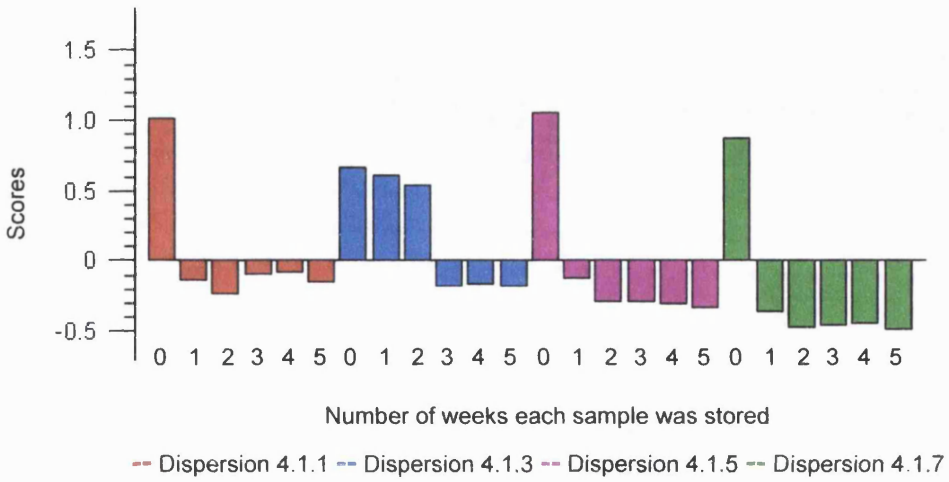

Figure 6.22 The PC 1 scores plot for all of the dispersion samples containing 60% griseofulvin by weight stored at 50 °C and 30% RH.

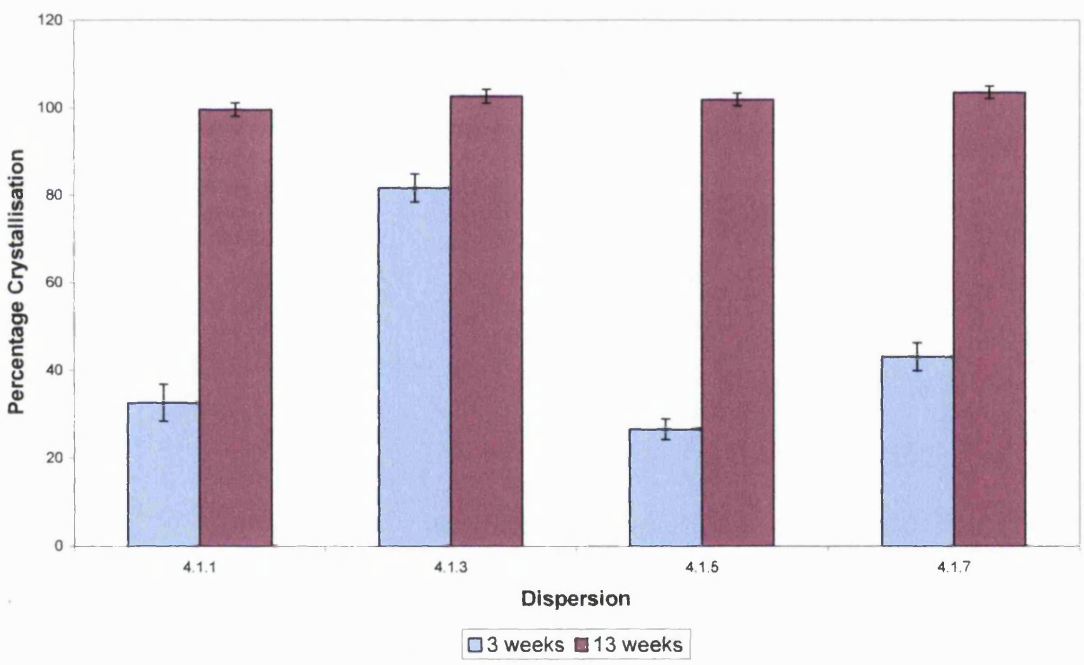

Figure 6.23 The crystallisation of griseofulvin dispersions stored at 50 °C and 30% RH for 13 weeks. This is a reproduction of some of the data in Figure 4.13.

6.3.4 A COMPARISON OF THE CRYSTALLISATION OF GRISEOFULVIN FROM SOLID DISPERSIONS STORED UNDER DIFFERENT CONDITIONS

In order to investigate the conditions under which crystallisation occurred in each of the samples it was decided that the individual dispersions should be investigated across all storage conditions. One would expect to see larger changes in the scores plots occurring under harsher conditions. This would also provide evidence that NIRS was detecting the crystallisation of the dispersions and one would also be able to examine the effect that water was having on the stability of the dispersions.

The scores plot for Dispersion 4.1.1 (binary) when stored under all the different conditions is shown in Figure 6.24, whilst Figure 6.25 contains the loadings plot for all the conditions which is similar to the SD-C plot. As expected the dispersion was most stable when stored at room temperature 0% RH. Dispersion 4.1.1 seemed to be less affected by low levels of humidity than an increase in temperature.

In Chapter 4 the pure PVP was exposed to a DVS cycle which included 5 h at 30% RH absorbed 10% of its own weight in water (Figure 4.18). As there was no possibility of hydrogen bonding between PVP and griseofulvin it was expected that Dispersion 4.1.1 would absorb a significant amount of water when stored at 30% RH. According to TGA the residual solvent following spray drying could have made up around 2% of the weight of the dispersion (Table 4.4), and at 0% RH this was probably removed fairly rapidly.

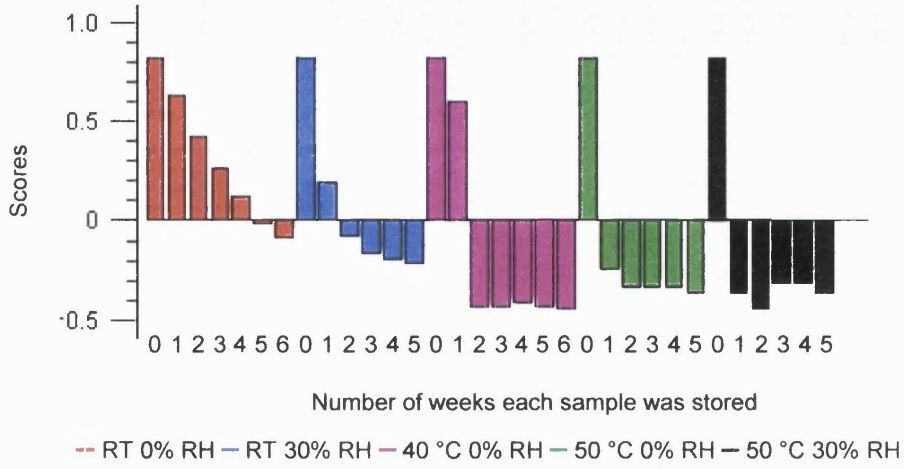

Figure 6.24 The PC 1 scores plot for Dispersion 4.1.1 stored at all conditions where RT = room temperature.

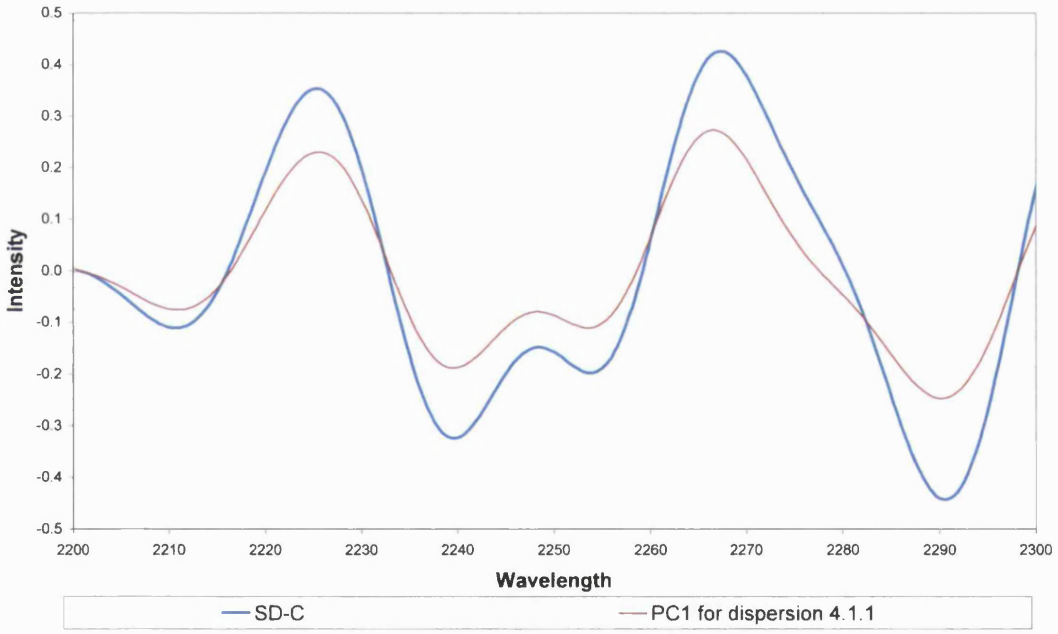

Figure 6.25 The PC 1 loadings plot for Dispersion 4.1.1 (binary) when all the sample sets from each storage condition were combined alongside the SD-C plot covering the wavelengths 2200-2300 nm.

Figure 6.26 shows the scores plot for Dispersion 4.1.3 (PAA). The relative rates of crystallisation were broadly the same as those observed for the Dispersion 4.1.1 (binary). Interesting, at 50 °C, Dispersion 4.1.3 would appear to have been more stable when stored at 30% RH than when stored at 0% RH. The two samples were stored in the same oven discounting the possibility of temperature differences. The glass

desiccators used were identical as were the 4 ml Waters vials. There are no convincing hypotheses; it may be that PAA was predominately interacting with the PVP through hydrogen bonding and the presence of water separated the two permitting an increased level of interaction between the PAA and the griseofulvin. The only slight difference that was detected was a degree of physical hardening and coarsening of the samples stored at 30% RH.

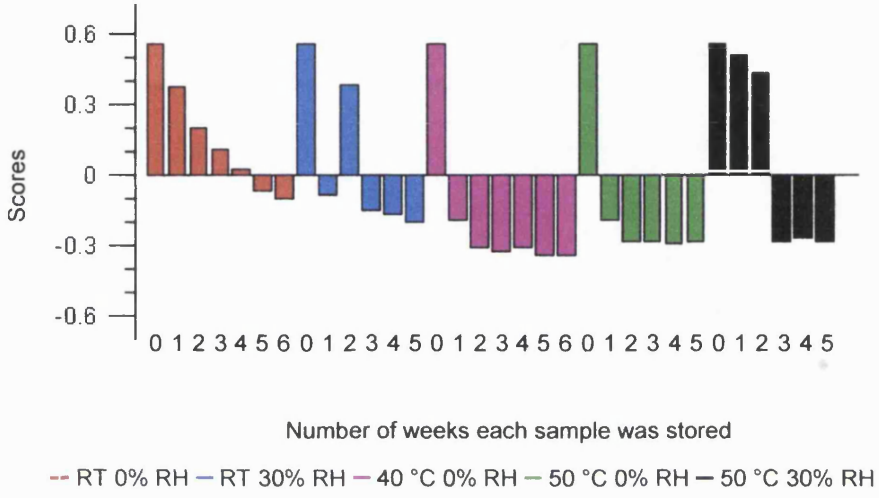

Figure 6.26 The PC 1 scores plot for Dispersion 4.1.3 (containing PAA), when all the sample sets covering each storage condition were combined.

The scores plot (Figure 6.27) for Dispersion 4.1.5 (PHPMA) is as one would expect. At room temperature there was no real trend in the data for the samples stored at either 0% RH or 30% RH, implying that under these conditions the dispersion was reasonably stable. An increase in temperature would appear to be detrimental to stability. There was a definite trend towards crystallisation when the dispersion was stored at 40 °C, 0% RH. At 50 °C, 0% RH considerably more crystallisation seemed to have occurred whilst storage at 30% RH resulted in a more rapid and complete level of crystallisation.

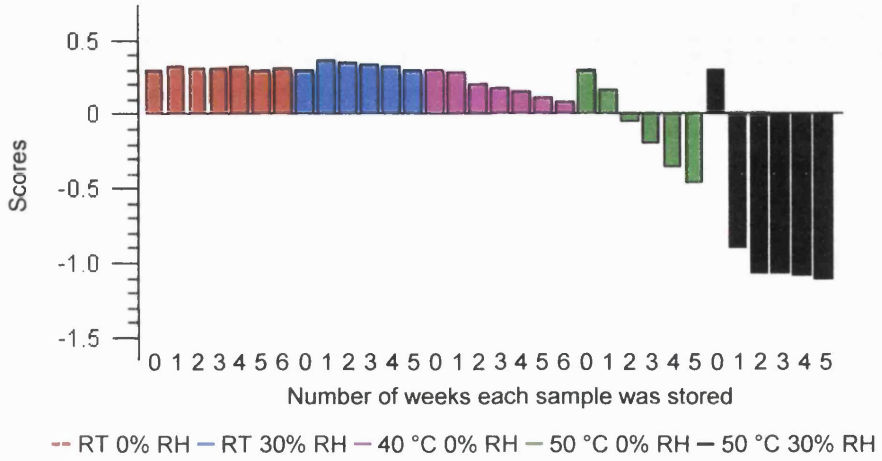

Figure 6.27 The PC 1 scores plot for Dispersion 4.1.5 (containing PHPMA), when all the sample sets covering each storage condition were combined.

Room temperature apart, Dispersion 4.1.7 (sucrose) showed a very similar (and rapid) rate of crystallisation regardless of the storage conditions. The sample stored at room temperature, 30% RH appears to be marginally more stable than the samples stored at higher temperatures whilst the sample stored at 0% RH is more stable still (Figure 6.28).

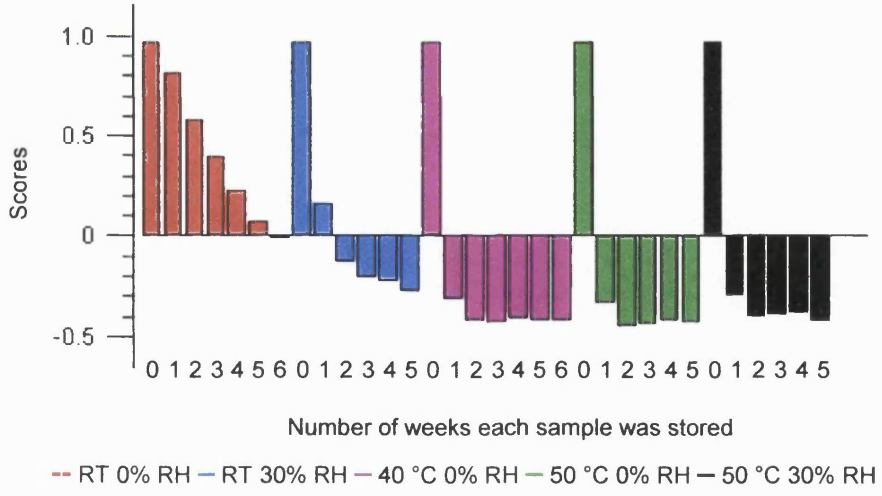

Figure 6.28 The PC 1 scores plot for Dispersion 4.1.7 (containing sucrose), when all the sample sets covering each storage condition were combined.

Figure 6.29 shows the PC 1 loadings plot for each of the above scores plots. These confirm that the different dispersions underwent the same crystallisation event. Figure 6.30 shows the PC 2 loadings plot for each of the different dispersions. There was no

real similarity between the different plots. This suggests that the PC 2 loadings plots were most probably noise or were related to the nature of the specific dispersions.

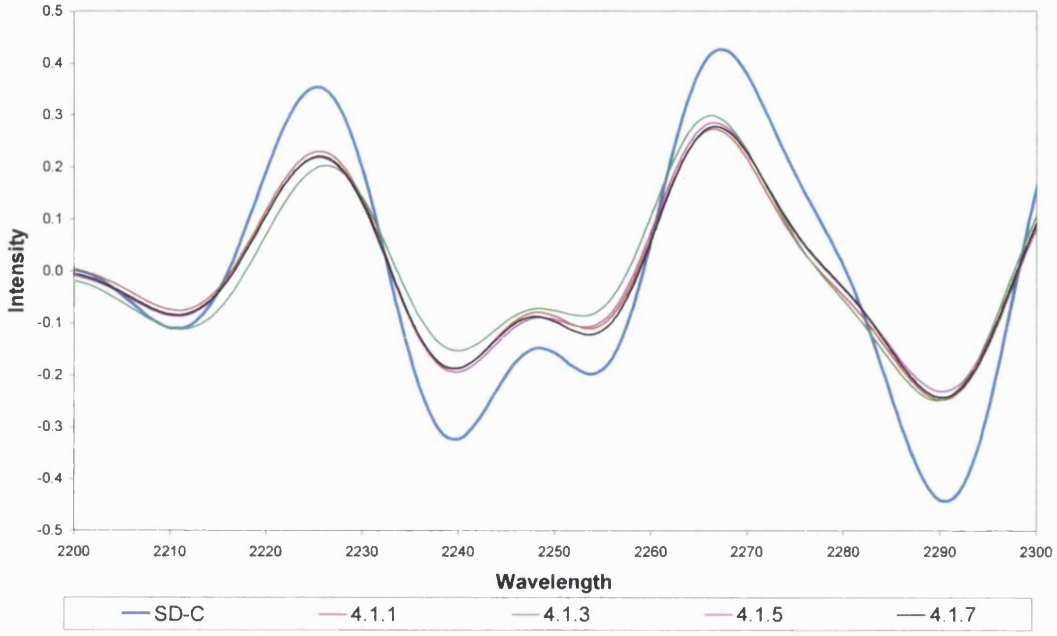

Figure 6.29 The PC 1 loadings plot for each of the individual dispersions stored at all conditions and compared with the SD-C plot.

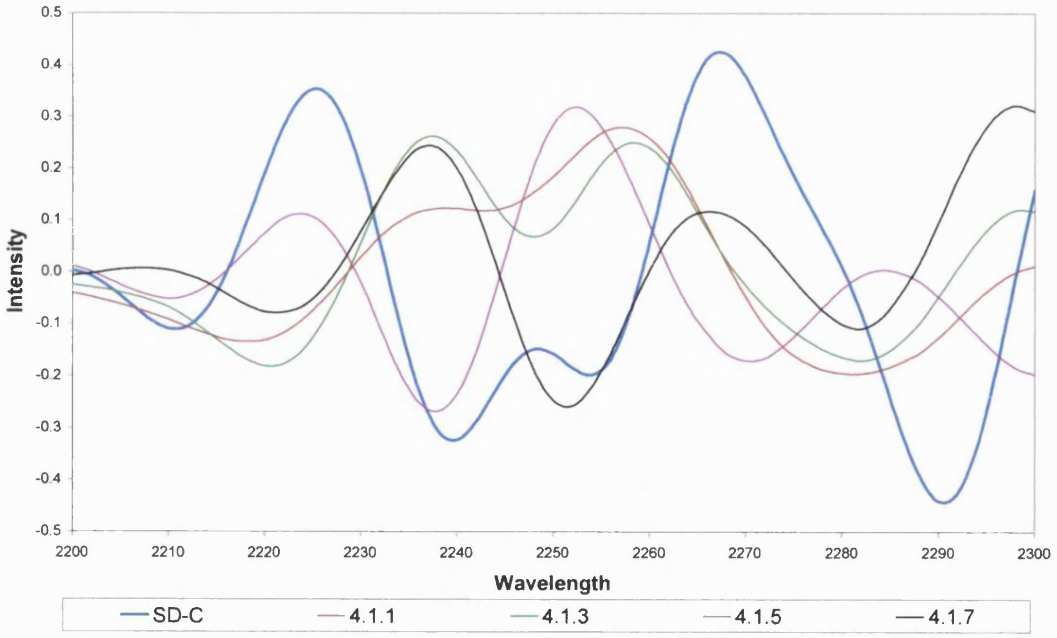

Figure 6.30 The PC 2 loadings plot for each of the individual dispersions stored at all conditions and compared with the SD-C plot.

6.3.5 A Comparison of all Polymers at all Conditions

Thus far the different dispersions have been examined together at a single condition. Each of the dispersions has also been examined when stored under different conditions and reasonable predictors for crystallisation have been found. It was decided that data for all the dispersions at all the conditions tested should be combined. This might prove to be less accurate but should it work it would prove to be a very rapid analytical tool. The PC 1 loadings plot showed the crystallisation double peak as expected (Figure 6.31). The PC 1 scores plot is also shown (Figure 6.32). Overall Dispersion 4.1.5 (PHPMA) was the most stable at all conditions. The only exception to this was the possibility that Dispersion 4.1.3 (PAA) was more stable at 50 °C and 30 % RH though the reason for this being the case was not clear.

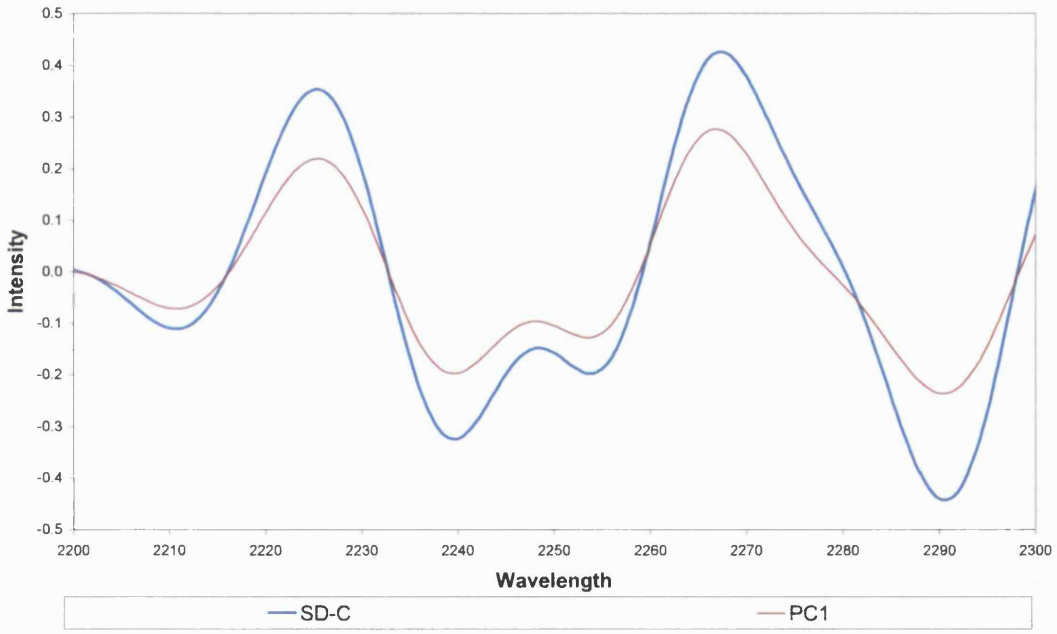

Figure 6.31 The PC 1 loadings plot for all dispersions stored at all conditions analysed as a single sample set and compared with the SD-C plot.

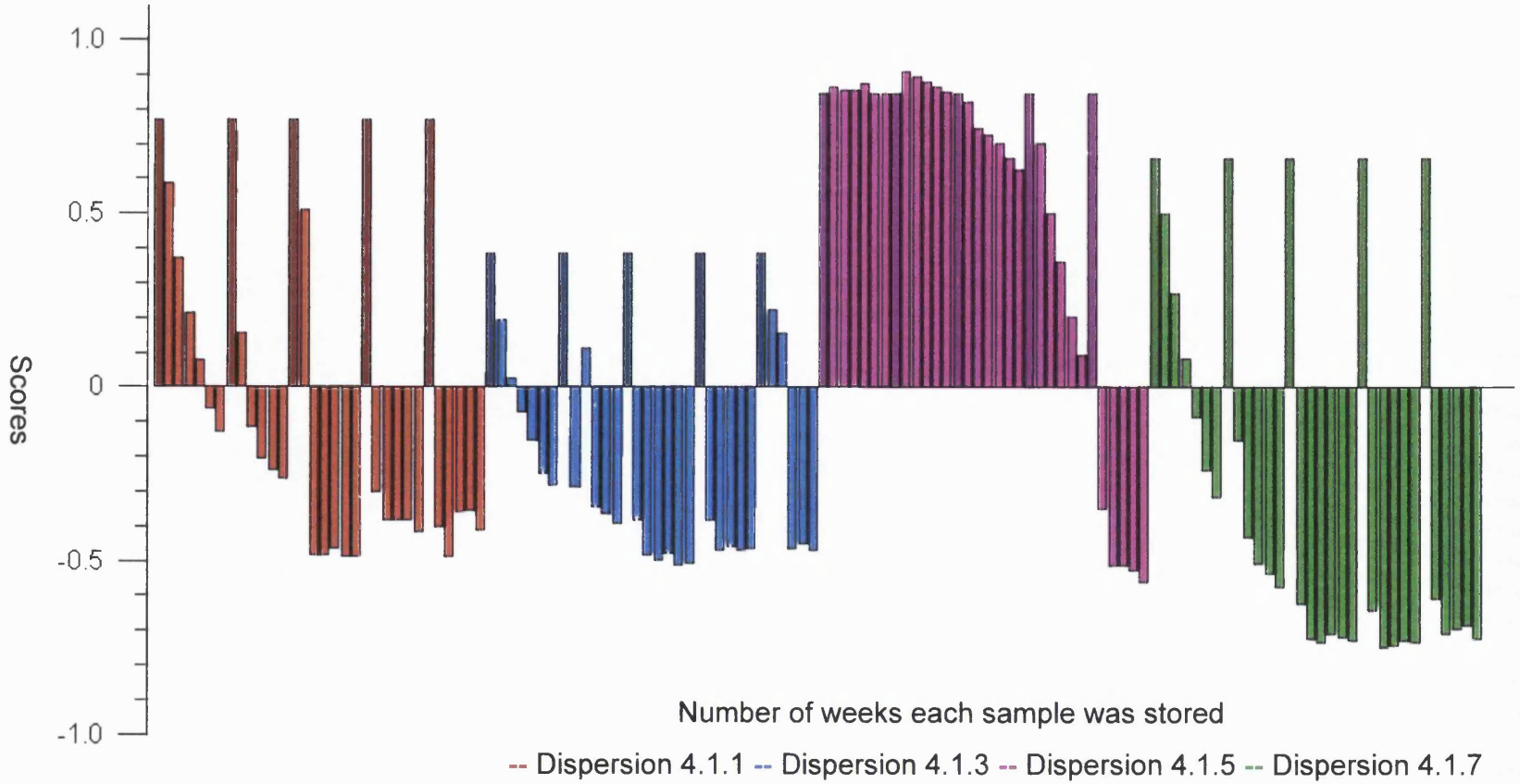

Figure 6.32 The PC-1 scores plot for all dispersions stored at all conditions analysed as a single sample set. The darker colour denotes a change in the conditions under which the dispersion was stored and these are ordered as follows; room temperature and 0% RH, room temperature and 30% RH, 40 °C and 0% RH, 50 °C and 0% and 50 °C and 30%.

6.3.6 The Scores and Loading Plots for a Single Dispersion Stored at a Single Condition

Selected scores and loading plots for individual dispersions stored at a single condition are presented in this section. This method of analysis was used to provide further support of the usefulness of PCA analysis of NIR spectroscopy as a tool for the detection of amorphous stability (or the lack of amorphous stability). Figures 6.33 and 6.34 show the scores and loading plots for Dispersion 4.1.1 (binary) stored at 50 °C and 0% RH. The loadings plots for samples stored at other conditions had a similar shape and this was clearly the crystallisation double peak. In general the scores plots were similar to the related sections in the scores plot for Dispersion 4.1.1 (binary) stored at all conditions (Figure 6.24). This proved to be the case for all the different dispersions stored under different conditions.

There were a few exceptions to this general rule. The loadings plot for Dispersion 4.1.3 (PAA) when stored at 50 °C/30 % RH is shown in Figure 6.35. This dispersion proved to be more stable when stored under humid conditions. The double peak in the PC 1 loadings plot was markedly less pronounced than in other dispersions and there were slight shifts in the other major peaks (around 2210 nm and 2290 nm). The PC 1 loadings plot for Dispersion 4.1.1 is shown in Figure 6.35 as a comparison.

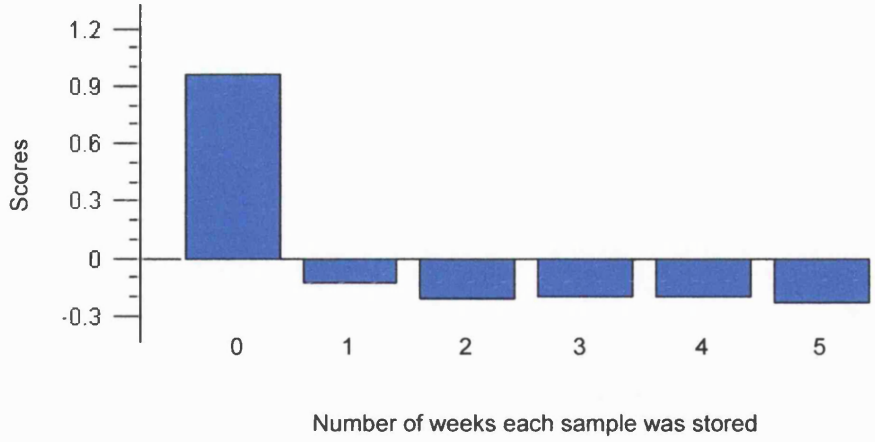

Figure 6.33 The PC 1 score plot for Dispersion 4.1.1 stored at 50 °C and 0% RH.

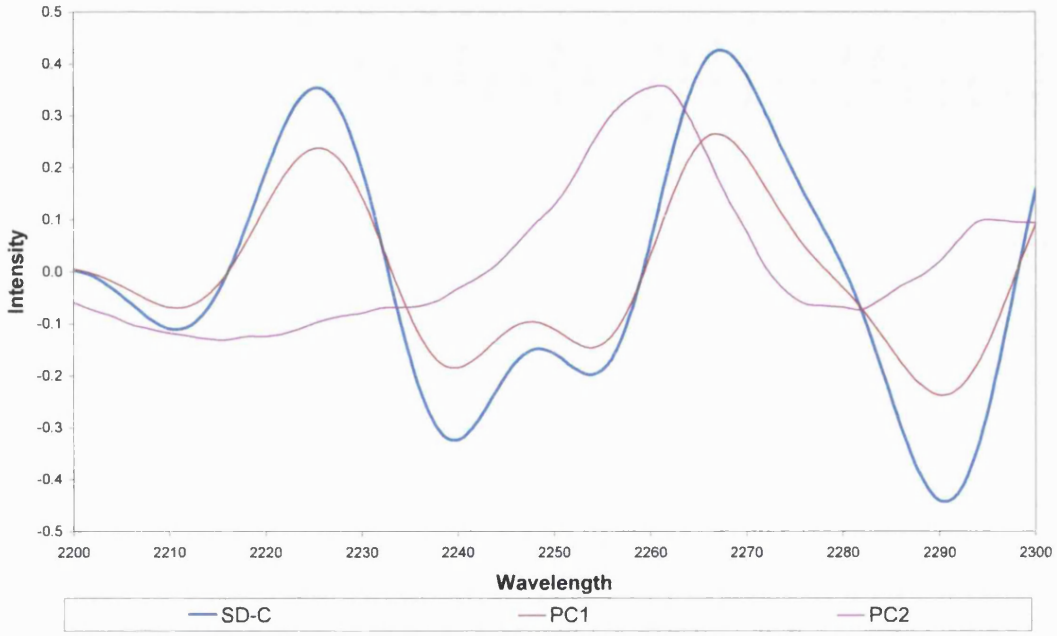

Figure 6.34 The PC 1 and PC 2 loadings plots for Dispersion 4.1.1 stored at 50 °C and 0% RH and compared with the SD-C plot.

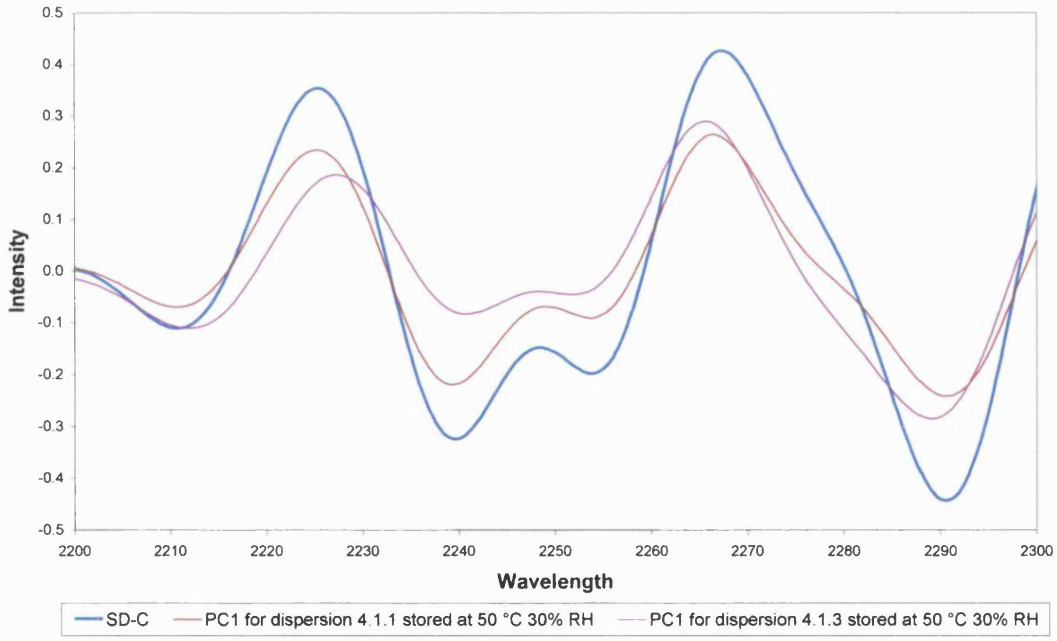

Figure 6.35 The PC 1 loadings plots for Dispersion 4.1.1 and Dispersion 4.1.3 stored at 50 °C and 30% RH and compared with the SD-C plot.

The PC 1 loadings plots for Dispersion 4.1.5 (PHPMA) when stored at room temperature, 0% RH and room temperature, 30% RH are shown in Figure 6.36. Neither of the plots was the same as that of crystallisation (SD-C) confirming that the dispersions were relatively stable. Figure 6.37a and 6.37b show the related scores plots

which suggest that at 0% RH the change was most probably noise. The scores plot for 30% RH (Figure 6.37b) showed a sudden change between week 0 and week 1 which was seen with the crystallisation scores plot for other dispersions at other conditions therefore it may have been a real event but given the loadings plot this was relatively unlikely.

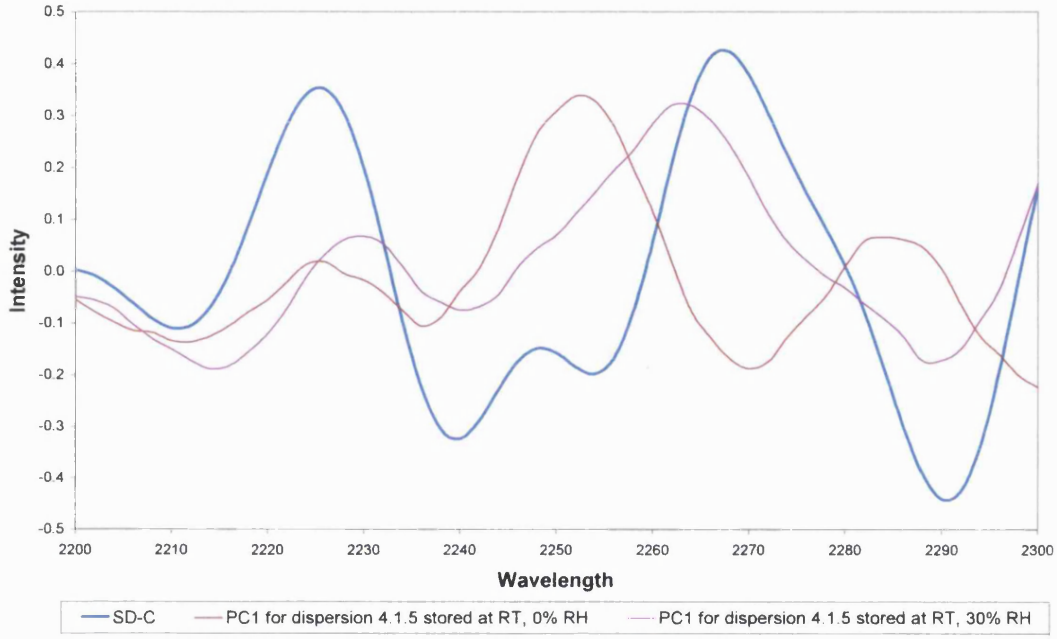

Figure 6.36 The PC 1 loadings plots for Dispersion 4.1.5 stored at room temperature and 0% RH and room temperature and at 30% RH, compared with the SD-C plot.

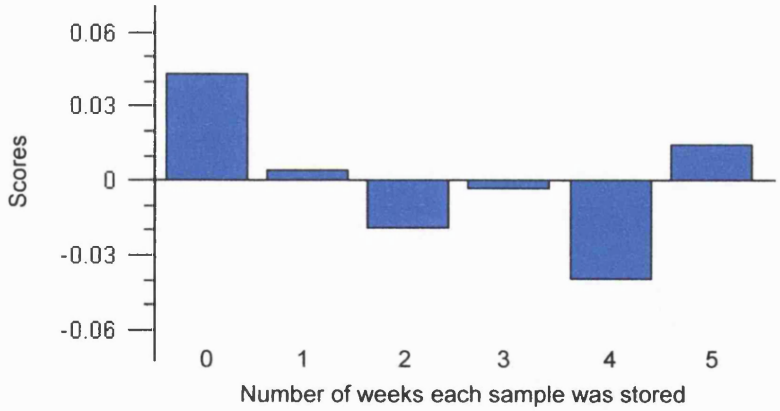

Figure 6.37a The PC 1 scores plot for Dispersion 4.1.5 stored at room temperature and 0% RH.

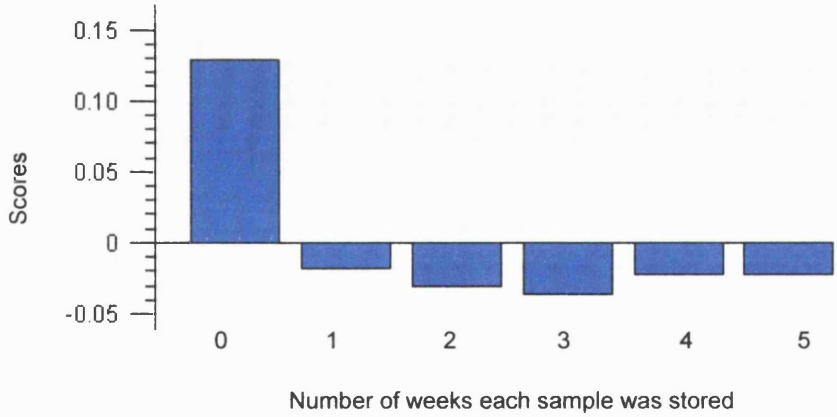

Figure 6.37b The PC 1 scores plot for Dispersion 4.1.5 stored at room temperature and 30% RH.

Figure 6.38 shows the PC 2 loadings plot for all the samples of Dispersion 4.1.5 stored at 0% RH (where it makes up 23% of the variance) and at 30% RH (22% of the variance). Whilst the PC 2 loadings plots do make up a significant percentage of the total change they lack consistency in terms of shape and when the scores plots were examined there were no distinct trends in the data (not shown). PC 2 is therefore most likely to be noise. When the samples of Dispersion 4.1.5 (PHPMA) were compared together the shape indicative of crystallisation of the loading plot was not present. Overall Dispersion 4.1.5 would appear to be remarkably stable. If it had not been analysed alongside the other dispersions than it would not have been possible to distinguish the crystallisation event from noise. This is of concern given that crystallisation was detected by XRPD. There is therefore a situation where some samples show almost complete crystallisation within a few weeks and some samples do not show any change. It is possible that NIR is sensitive to a more narrow band of change than XRPD.

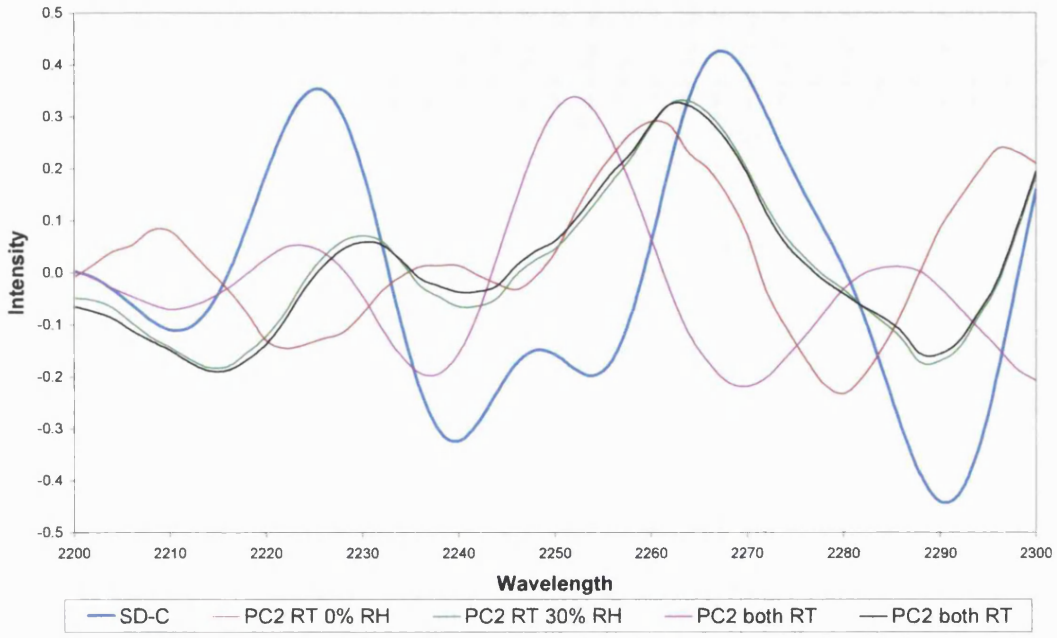

Figure 6.38 The PC 2 loadings plots for Dispersion 4.1.5 stored at room temperature and 0% RH and at 30% RH along with the PC 1 and PC 2 loadings plots for the combined room temperature conditions.

Overall there were no real patterns detected in the PC 2 plots for any of the dispersion samples and therefore these are most likely noise though one cannot discount the possibility that the different dispersions undergo very different changes on storage and these changes vary depending on the conditions of storage.

6.3.7 Changes in the Water Region of the NIR Spectra

It was postulated that the changes in the free and bound water in samples would differ depending on the conditions under which they were stored, specifically under 0% RH or 30% RH. In a moisture rich environment, the polymer, the drug or the drug polymer complex could absorb the water from the atmosphere. Understanding the way in which water alters stability may be useful. The region of NIR most associated with water covers the wavelengths 1800-2000 nm (Seisler *et al.*, 2002). PVP as supplied was placed in a DVS-NIR system, dried at 0 % RH for 6 h and subsequently exposed to 30% RH for 6 h, before being dried again for 6 h. The water gain and loss of the PVP was recorded as a percentage of dry weight (Figure 6.39a, also shown in Figure 4.16). Selected NIR spectra of the PVP taken during this DVS experiment are presented in Figure 6.39b. The major peak changes are to be found at 1890 nm, 1910 nm and 1932

nm. The NIR spectra for the four excipients were presented at the start of this section in Figure 6.5b.

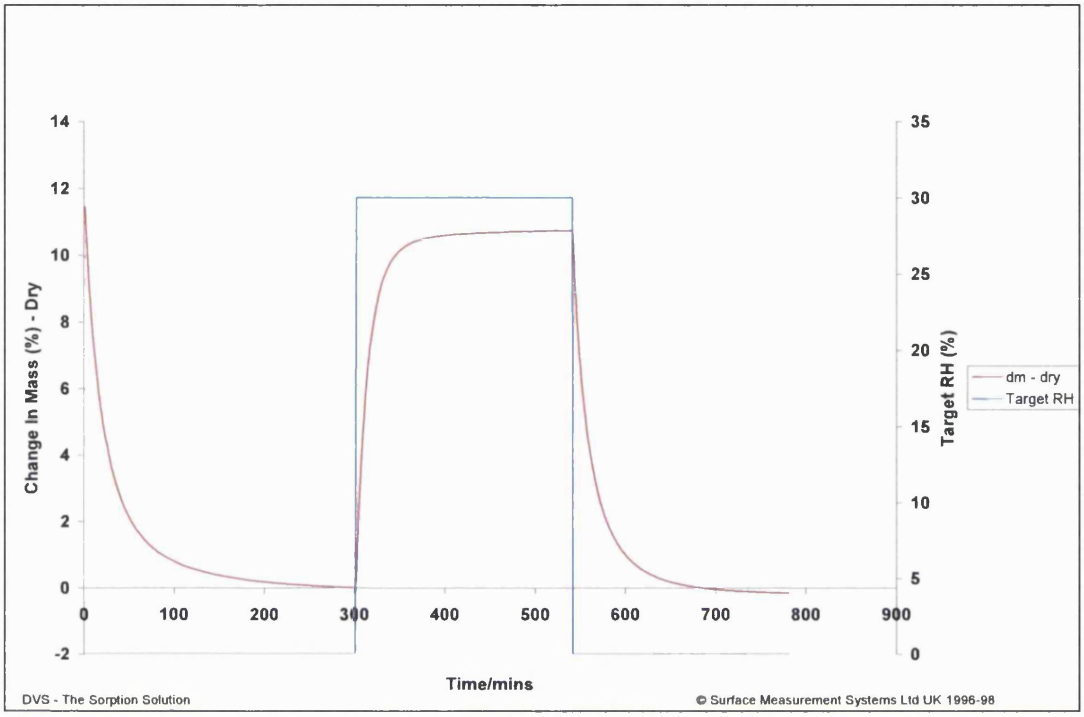

Figure 6.39a The water uptake of PVP under a relative humidity of 30%. This is a reproduction of the data in Figure 4.16.

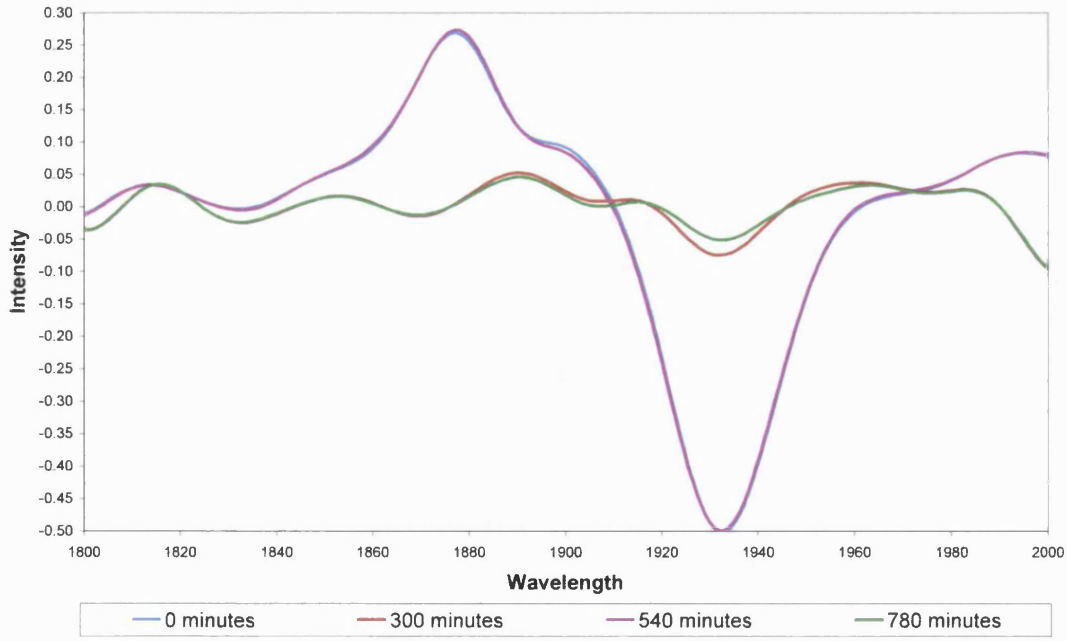

Figure 6.39b Selected NIR spectra of PVP covering water changes in the spectra due to water uptake and water loss at 30% RH.

The NIR spectra for Dispersion 4.1.1 (binary) stored at 50 °C, 0% RH and 50 °C, 30% RH show that even without moisture (at 0% RH) there were peak changes at 1880nm and 1932nm, although these were not as pronounced as the changes seen under more humid conditions (30% RH). A shoulder can be seen at 1910nm (Figure 6.40). These peaks were also seen in Dispersion 4.1.3 (PAA), Dispersion 4.1.5 (PHPMA) and Dispersion 4.1.7 (sucrose) samples, although the shoulder at 1910nm is less pronounced for the Dispersion 4.1.3 and Dispersion 4.1.7 (Figures 6.39-6.43).

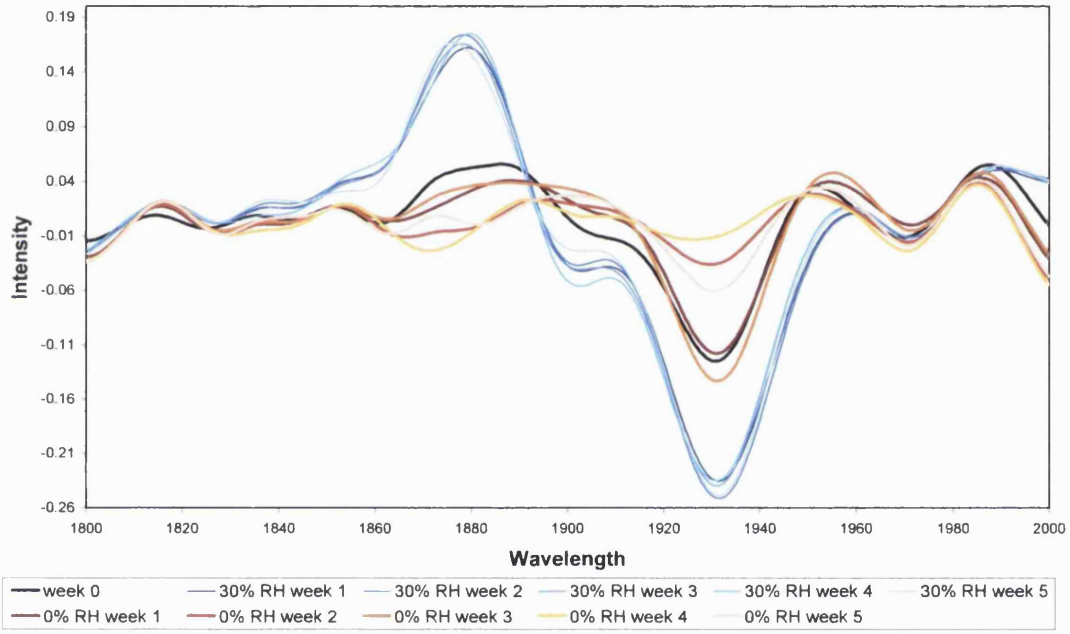

Figure 6.40 NIR spectra of Dispersion 4.1.1 covering water changes in the spectra occurring when stored at 0% RH and 30% RH.

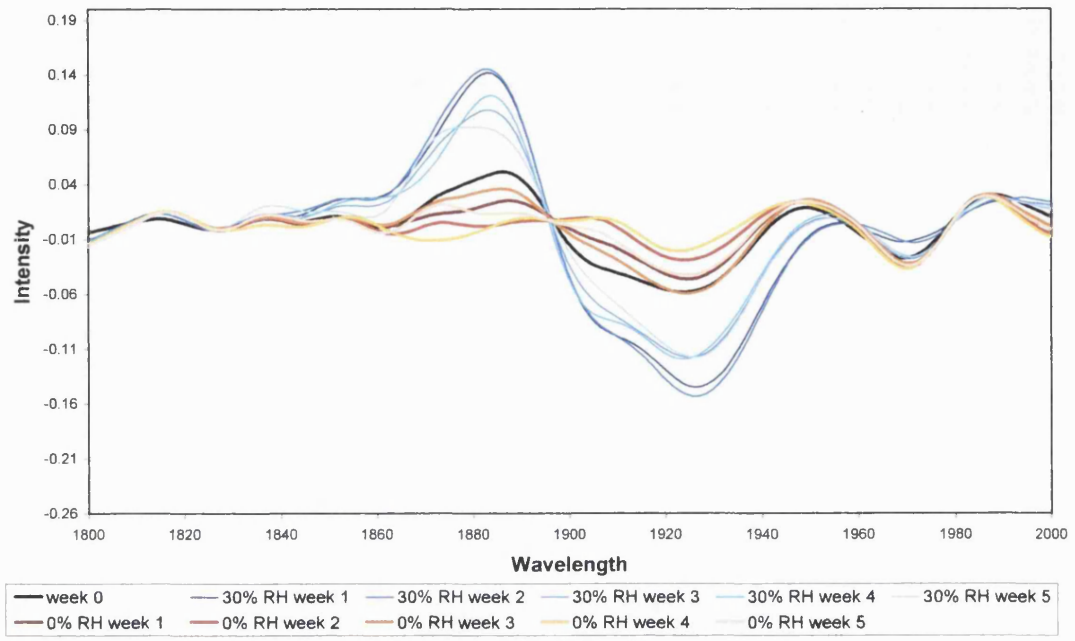

Figure 6.41 NIR spectra of Dispersion 4.1.3 covering water changes in the spectra occurring when stored at 0% RH and 30% RH.

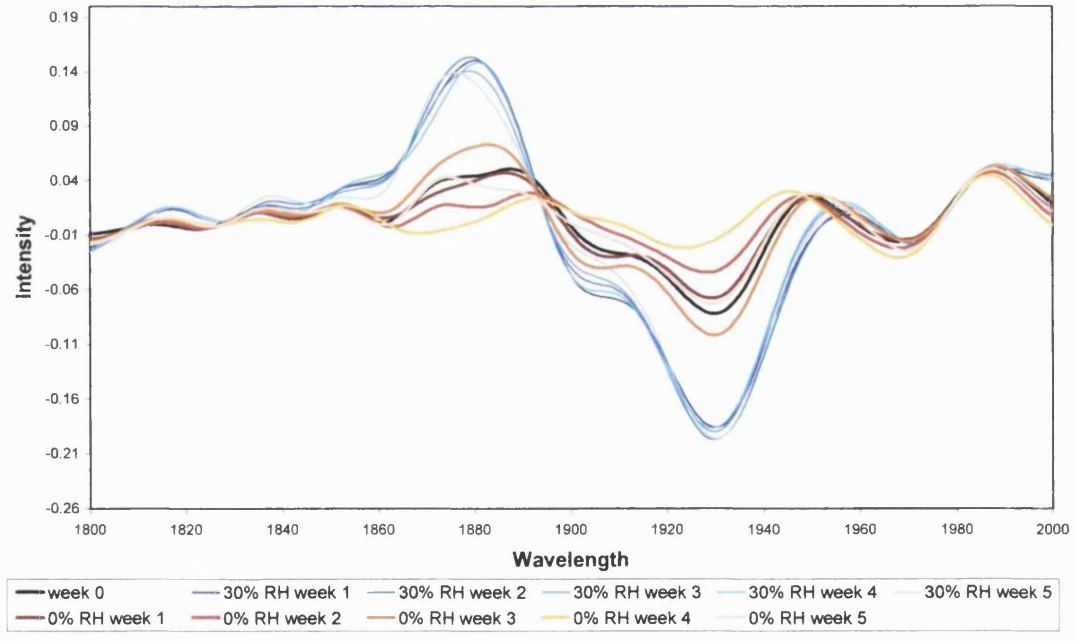

Figure 6.42 NIR spectra of Dispersion 4.1.5 covering water changes in the spectra occurring when stored at 0% RH and 30% RH.

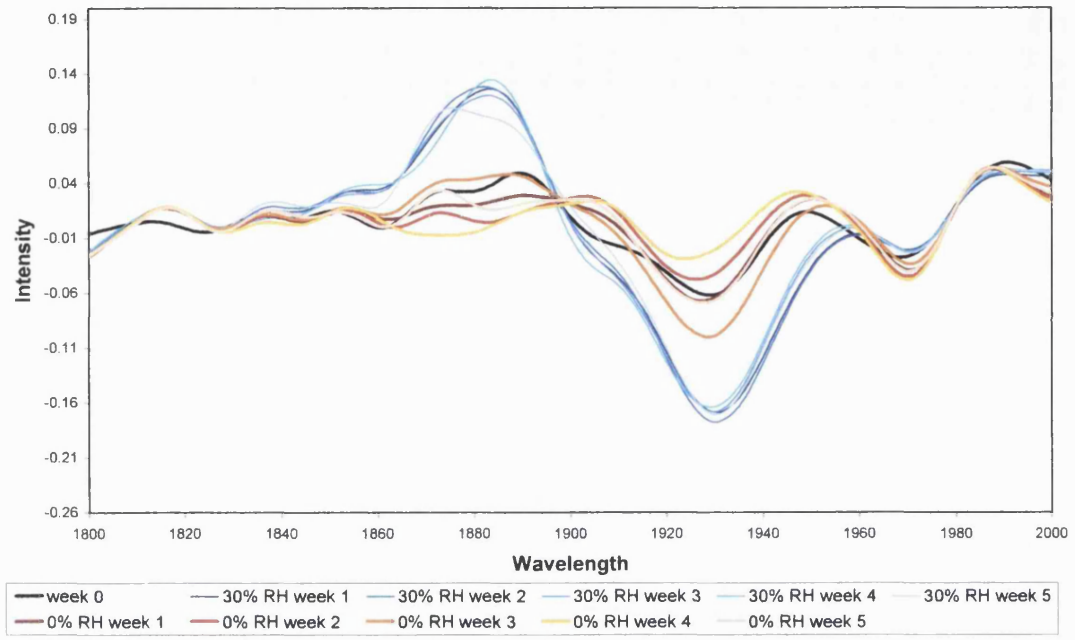

Figure 6.43 NIR spectra of Dispersion 4.1.7 covering water changes in the spectra occurring when stored at 0% RH and 30% RH.

A PC 1 loadings plot between 1800-2000 nm was produced for each of the dispersions covering only those samples stored at 30% RH (both room temperature and 50 °C). These were then plotted against the SD-C plot and a PC 1 loadings plot of PVP run in the DVS-NIR (Figure 6.44). There were some similarities between the PC 1 loadings plots for the dispersions and the SD-C plot. Both contained a peak at around 1880 nm. This was most probably due to the interaction between water and PVP as the PC 1 loadings plot for PVP also contains a peak at around 1880 nm. Dispersion 4.1.1 (binary), Dispersion 4.1.3 (PAA) and Dispersion 4.1.5 (PHPMA) all had peaks at around 1900 nm as did the SD-C plot whilst the PC 1 loadings plot for Dispersion 4.1.7 (sucrose) had a shoulder at the same point. Again however the PC 1 loadings plot for the DVS-PVP also had a small peak at around the same point. At 1936 nm there were peaks in Dispersion 4.1.1 (binary), Dispersion 4.1.3 (PAA) and Dispersion 4.1.7 (sucrose). Dispersion 4.1.5 (PHPMA) had a shoulder at the same point. This peak could have been related to changes in the PVP or in the griseofulvin.

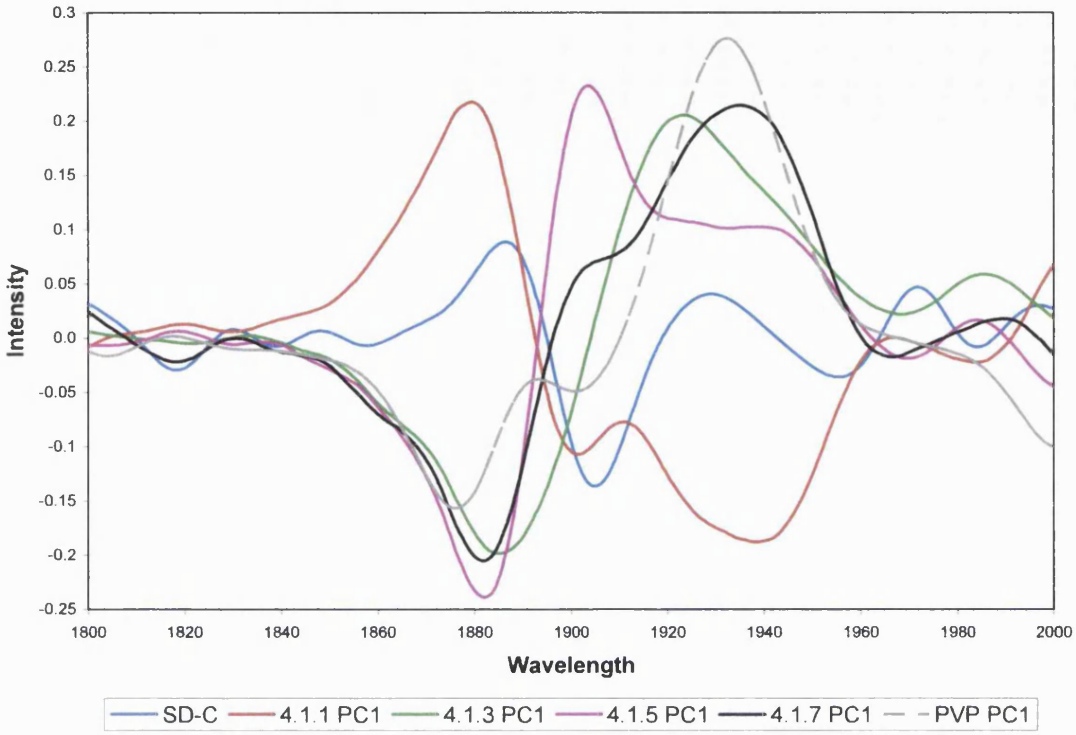

Figure 6.44 The PC 1 loadings plots for dispersions stored at 30% RH covering the water region (1800-2000nm).

The PC 1 scores plots for each of the dispersion samples stored at 30% RH is presented in Figure 6.45a-d. There were changes in the water content during storage with the greatest change occurring after the first week. Work performed on dispersions containing 10% and 20% reserpine in PVP in which the dispersions were stored at 20 °C and 50% RH showed that both uptake of water and T_g displayed a level after one day of exposure to 50% RH which did not alter over time. The equilibrium water content in the dispersions was lower than in pure PVP, around 17% for the dispersion and 21% for PVP (Jans-Frontini and Mielck, 1996).

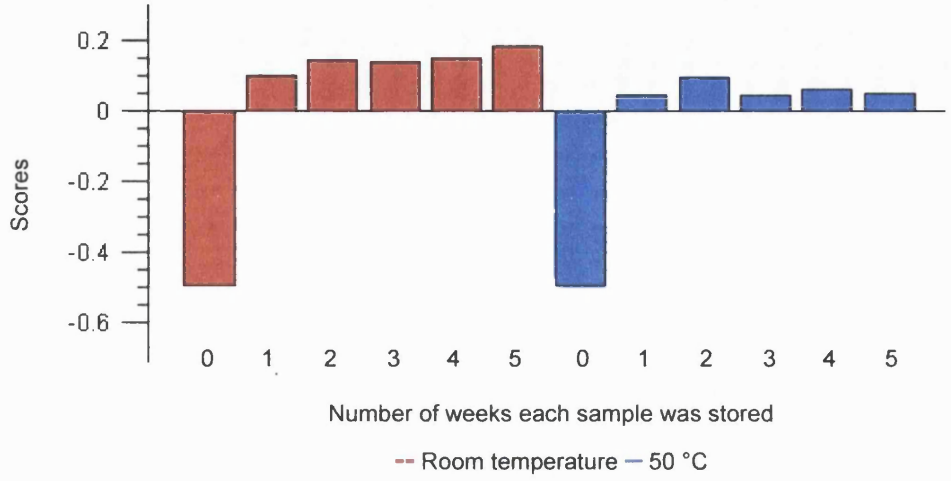

Figure 6.45a The PC 1 scores plot for Dispersion 4.1.1 stored at 30% RH covering the water region (1800-2000nm).

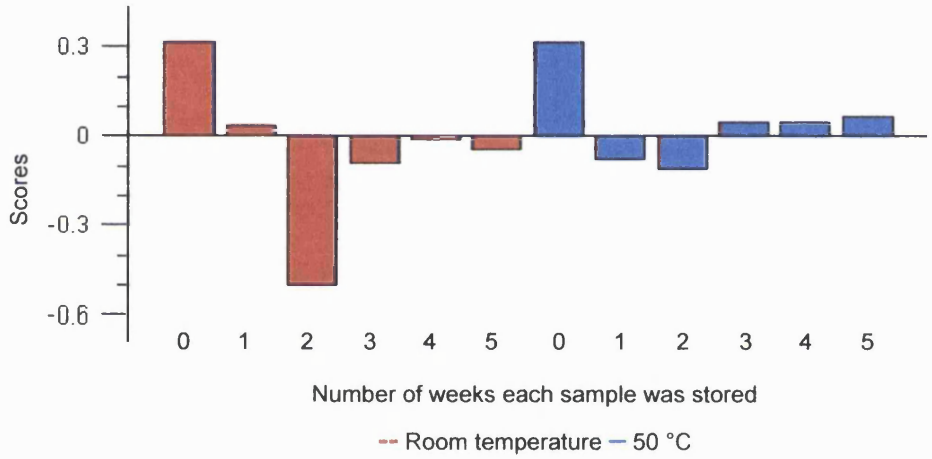

Figure 6.45b The PC 1 scores plot for Dispersion 4.1.3 stored at 30% RH covering the water region (1800-2000nm).

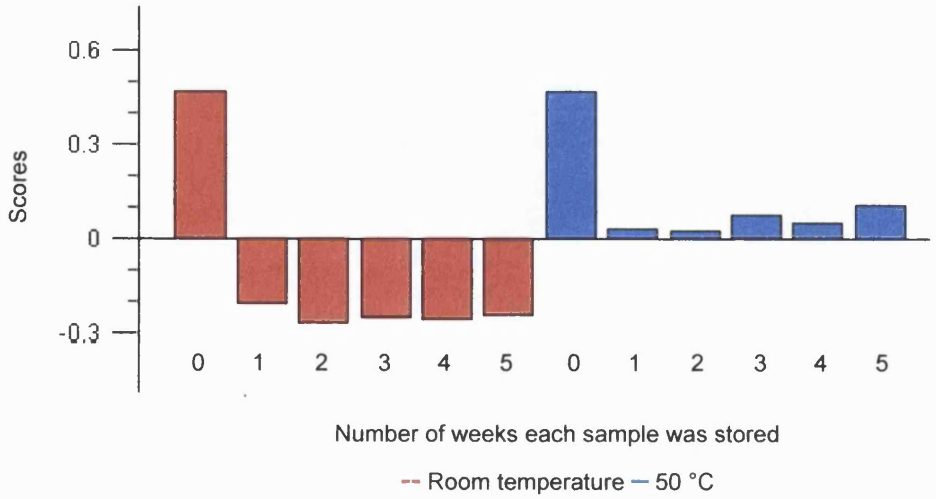

Figure 6.45c The PC 1 scores plot for Dispersion 4.1.5 stored at 30% RH covering the water region (1800-2000nm).

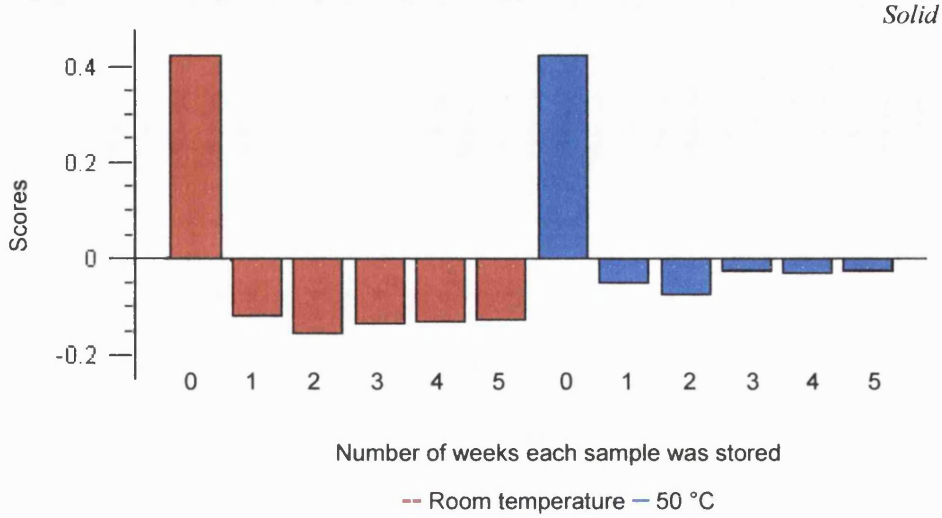

Figure 6.45d The PC 1 scores plot for Dispersion 4.1.7 stored at 30% RH covering the water region (1800-2000nm).

The PC 1 loadings plot for all the dispersions stored under all temperatures at 0% RH and the PC 1 loadings plot for all the dispersions stored under all temperatures at 30% RH were reasonably similar. Unfortunately bar a peak between 1900-1910 nm neither followed the SD-C plot (Figure 6.46). One would have expected there to be a decrease in the amount of water present in the dispersions as the griseofulvin crystallised when the dispersions were stored at 0% RH. The PC 1 scores plot for those samples stored at 30% RH would suggest that the spectral change was related to a sudden influx of water (Figure 6.47) yet the scores plot for those samples stored at 0% RH was completely random (not shown). The crystallisation of griseofulvin from these dispersions at 0% RH was therefore unlikely to be linked to water content. This was unexpected given the T_g observed in the dispersions, which suggested there must be some degree of plasticisation facilitating crystallisation.

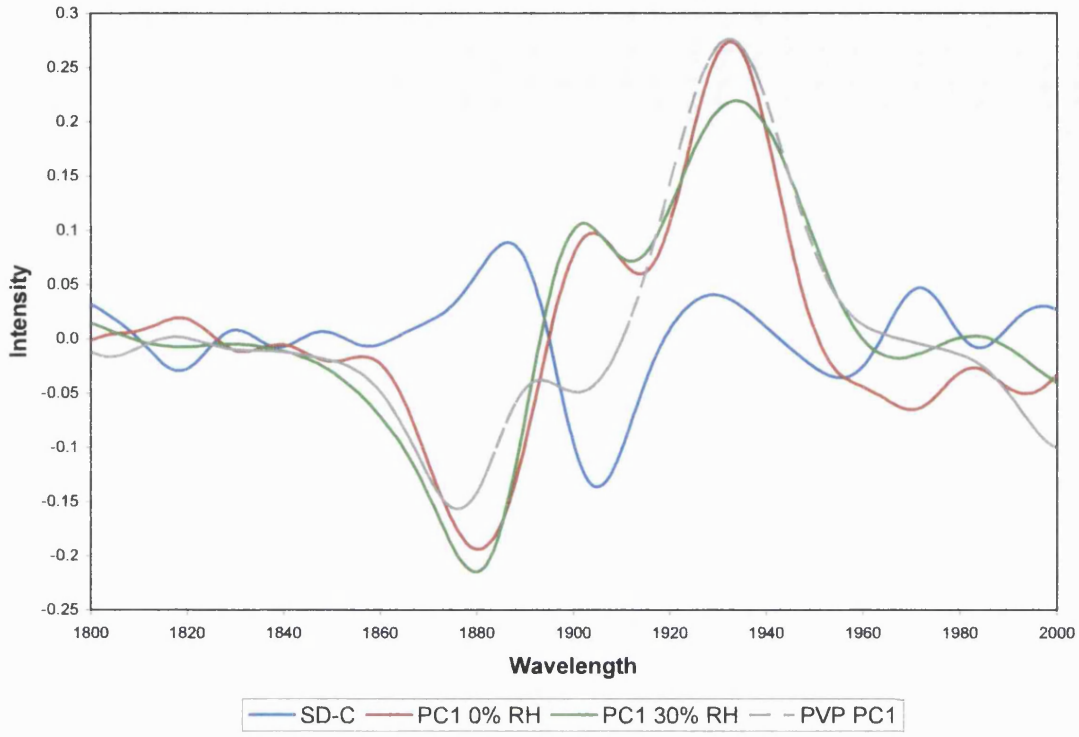

Figure 6.46 The PC-1 loadings plots for all dispersions stored at 0% and 30% RH analysed as two separate sample sets and compared with the SD-C plot.

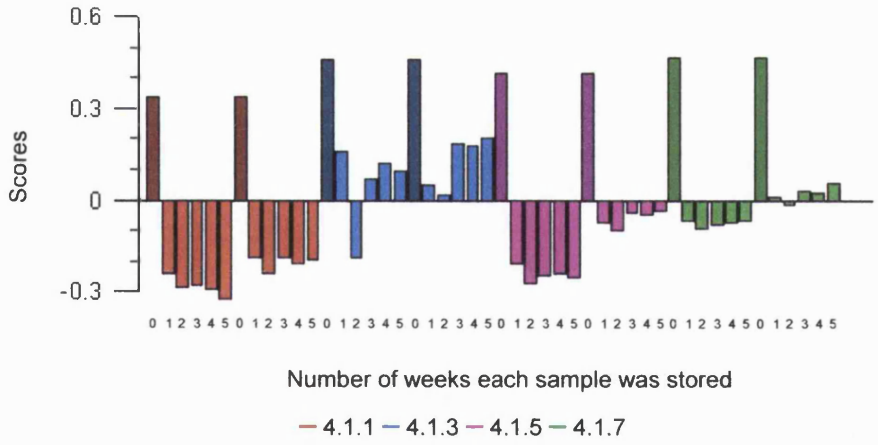

Figure 6.47 The PC-1 scores plot for all dispersions stored at 30% RH analysed as a single sample set. The darker colour denotes a change in the conditions under which the dispersion was stored and these are ordered as follows; room temperature and 30% RH and 50 °C and 30% RH.

6.3.8 The Crystallisation of Dispersions Containing Griseofulvin 40% w/w

Thus far the data analysis has focused on dispersions containing 60% griseofulvin by weight. Dispersions containing 40% griseofulvin by weight were also analysed using similar methods. PC 1 loadings plots for the 40% griseofulvin dispersions stored at each temperature and each relative humidity covering the wavelengths 2200-2300 nm had a very similar pattern to those dispersions containing 60% griseofulvin by weight and to that of the SD-C plot (Figure 6.48). This suggests that crystallisation was the main contributor to change in the samples. Somewhat surprisingly, the PC 1 loadings plot for dispersions stored at 50 °C and 30% RH looked like an inversion of the other loadings plots. This could still imply crystallisation if the scores plot showed increasing values.

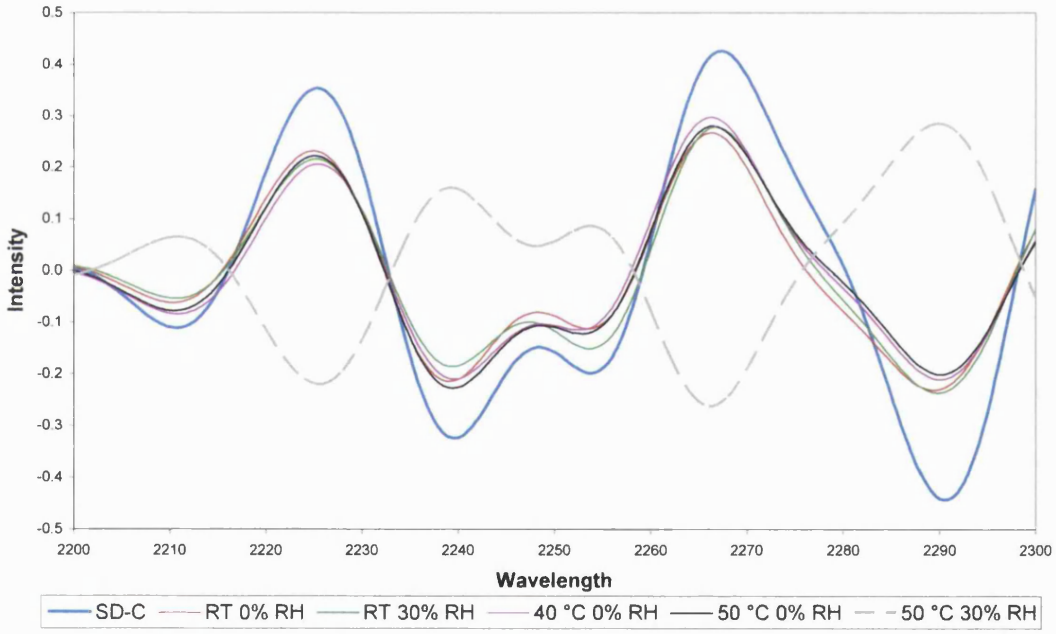

Figure 6.48 Loadings plots for all dispersion samples containing 40% griseofulvin by weight separated by storage conditions.

The PC 1 scores plots (Figures 6.49-6.53) show that very little change occurred in the samples as a function of time, especially at room temperature. The scores plot for Dispersion 4.1.2 (binary) showed some signs of crystallisation at 40 °C and 0% RH, but the sudden changes which occurred over the first three points suggest that any such change could be due to noise (Figure 6.51). The scores plot for storage at 50 °C, 0% RH shows there is a definite trend for both Dispersion 4.1.2 and Dispersion 4.1.6

(PHPMA) (Figure 6.52). This suggests that these two dispersions were crystallising and that Dispersion 4.1.4 (PAA) and Dispersion 4.1.8 (sucrose) were not. Figure 6.54 shows the crystallisation of griseofulvin from the dispersions when stored at 50 °C and 0% RH as detected by XRPD. This data was taken after 3 and 13 weeks of storage. The results would suggest that Dispersion 4.1.2 and Dispersion 4.1.6 were the more stable of the dispersions. At 50 °C and 30% RH there was also a trend towards crystallisation for Dispersion 4.1.2 and Dispersion 4.1.6 but the trend was comparable in size to the level of noise associated with Dispersion 4.1.4 (Figure 6.54). It should also be noted that whilst Dispersion 4.1.2 was observed (by XRPD) to be the least stable dispersion after both 3 and 13 weeks when stored at 50 °C, Dispersion 4.1.6 was observed to be the most stable (Figure 6.55). It is therefore difficult to make absolute statements about crystallisation in these dispersions under these conditions

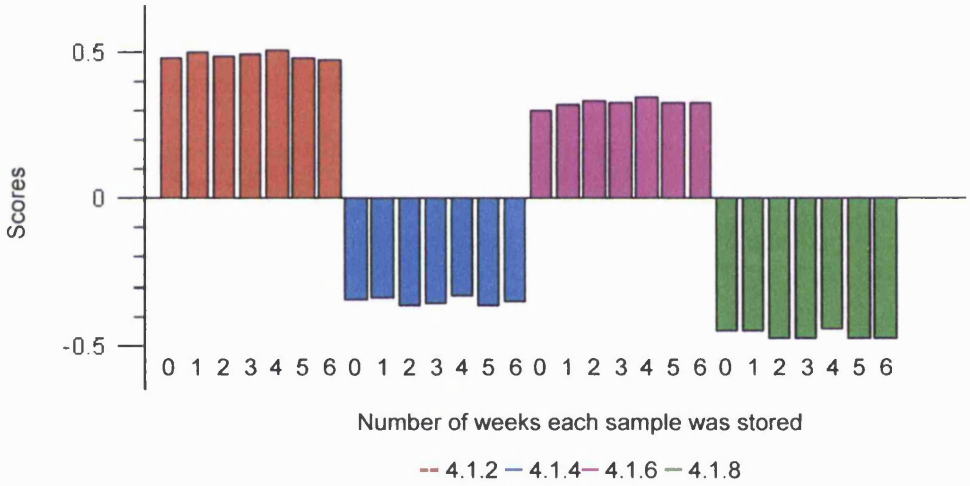

Figure 6.49 The PC-1 scores plot for all dispersions containing 40% griseofulvin stored at room temperature and 0% RH.

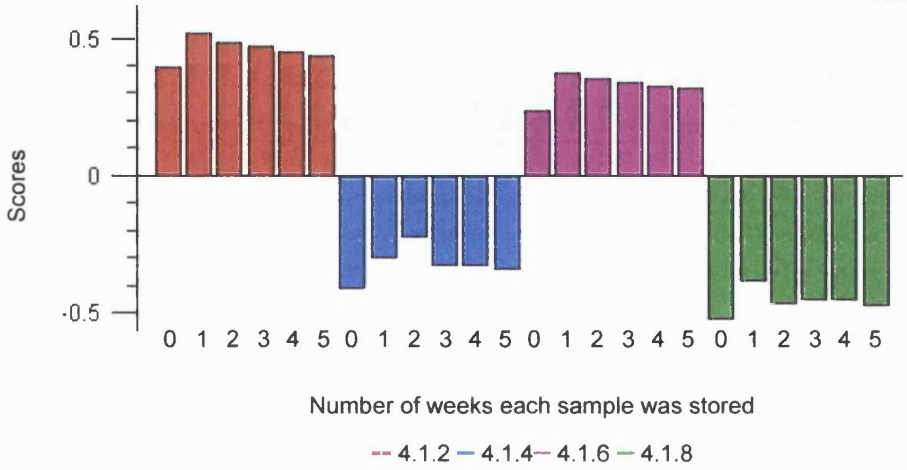

Figure 6.50 The PC-1 scores plot for all dispersions stored at room temperature and 30% RH.

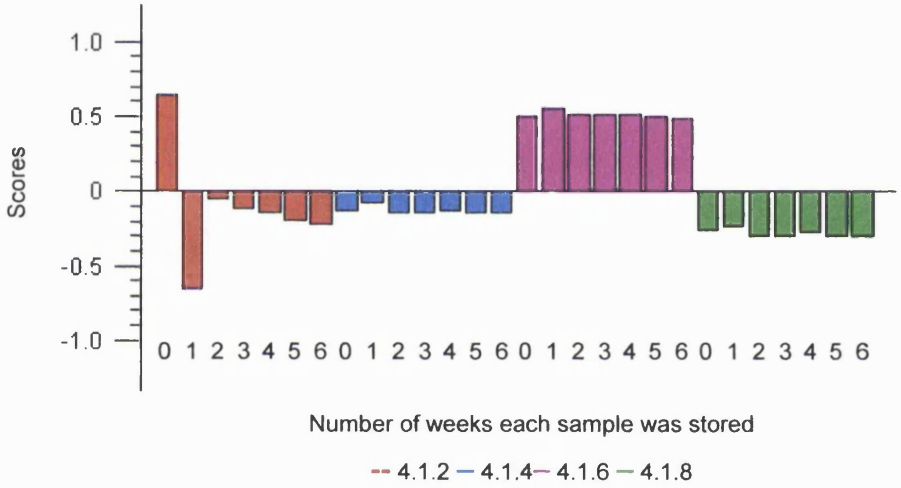

Figure 6.51 The PC-1 scores plot for all dispersions stored at 40 °C and 0% RH.

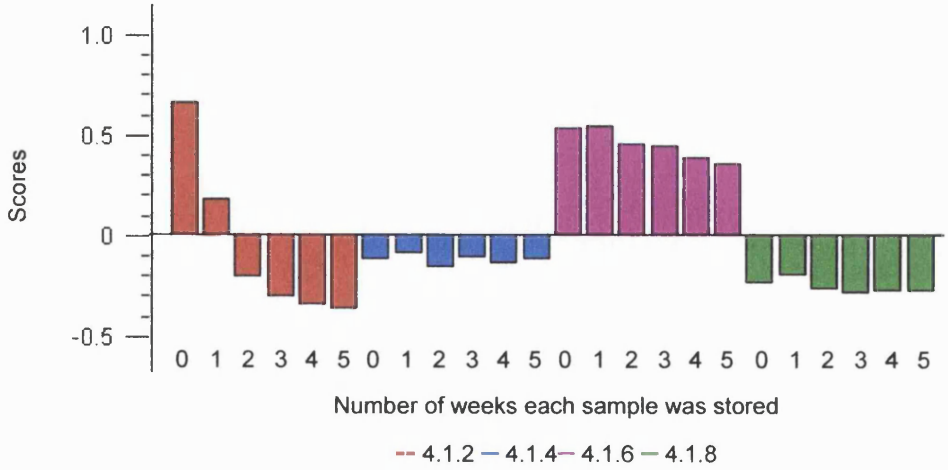

Figure 6.52 The PC-1 scores plot for all dispersions stored at 50 °C and 0% RH.

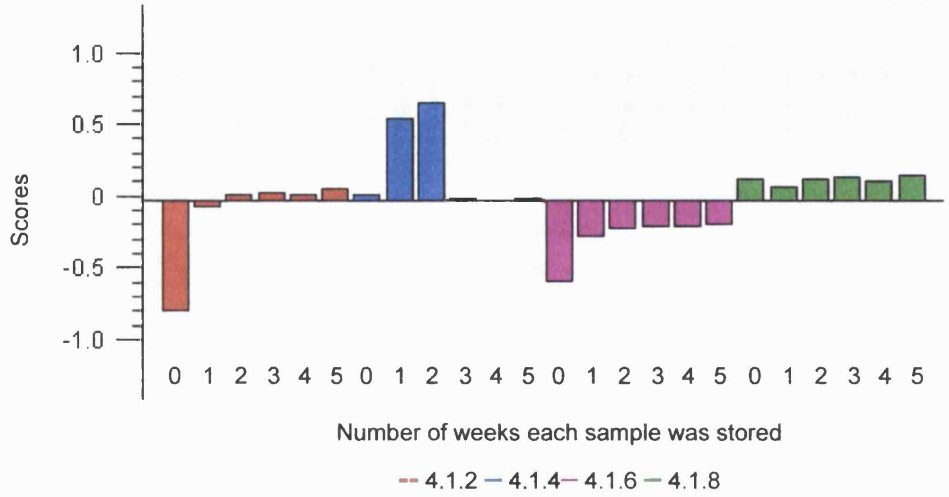

Figure 6.53 The PC-1 scores plot for all dispersions stored at 50 °C and 30% RH.

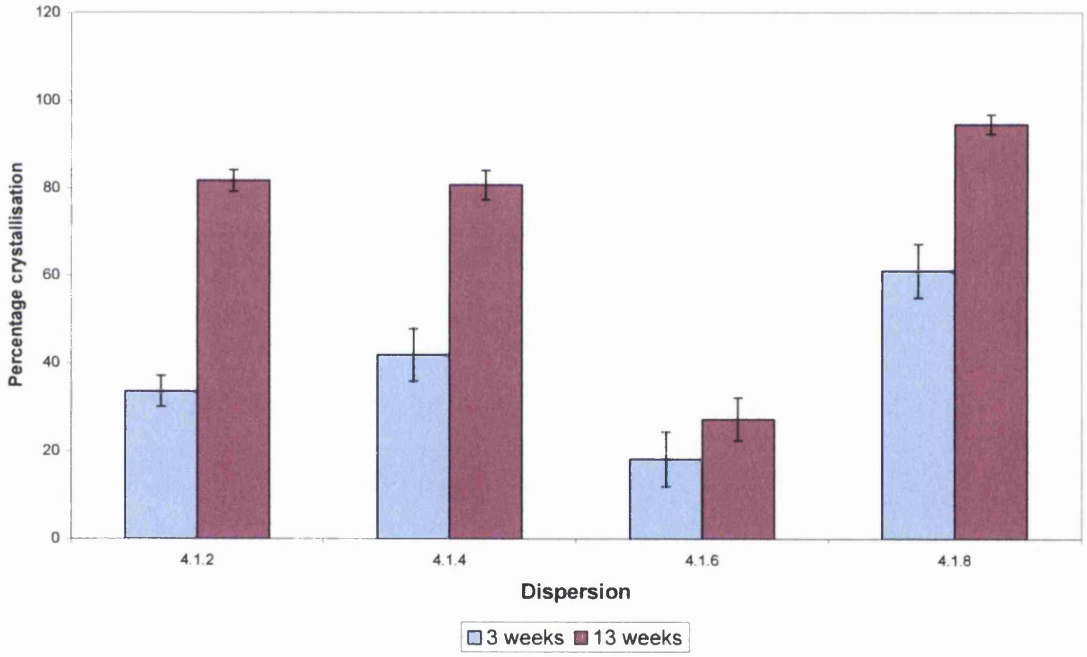

Figure 6.54 The crystallisation of griseofulvin dispersions stored at 50 °C and 0% RH for 13 weeks. This is a reproduction of some of the data in Figure 4.7.

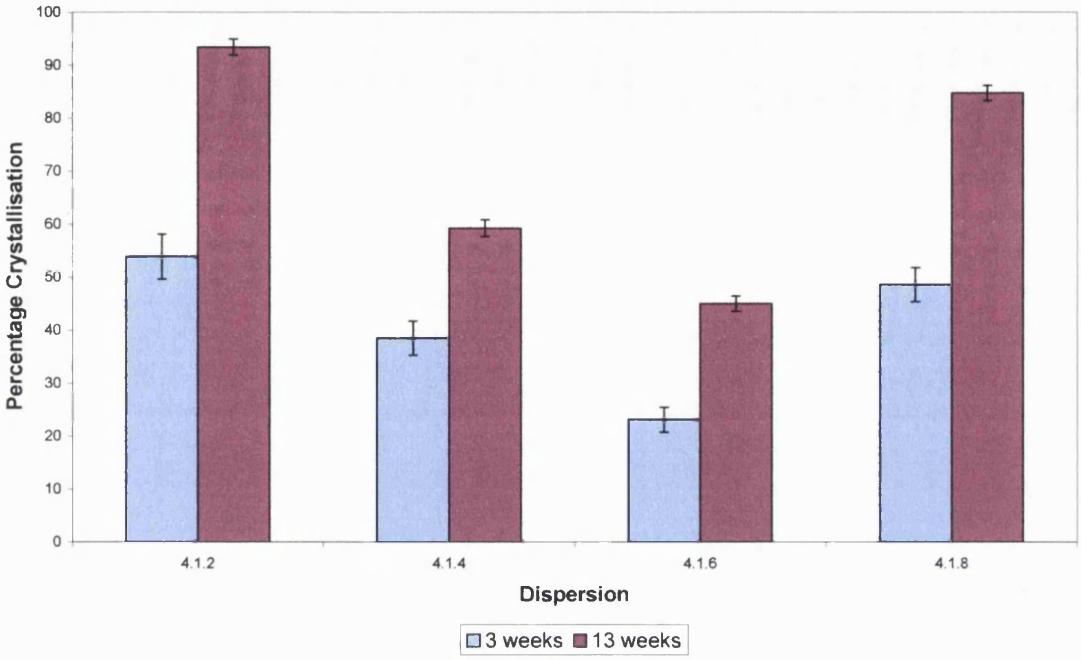

Figure 6.55 The crystallisation of griseofulvin dispersions stored at 50 °C and 30% RH for 13 weeks. This is a reproduction of some of the data in Figure 4.13.

The amorphous stability of each dispersion under varying conditions was then compared. The PC 1 loadings plots for the different dispersions are shown in Figure 6.56. The loadings were not all indicative of crystallisation, suggesting there may have been very little change over time. The loadings plots for Dispersion 4.1.2 (binary) and 4.1.6 (PHPMA) were of crystallisation, but the loadings plots for Dispersion 4.1.4 (PAA) and Dispersion 4.1.8 (sucrose) were different. As the loadings plot is dimensionless, the loadings plot for Dispersion 4.1.4 could have been an inverted crystallisation plot. The peaks between 2230-2260 were not as pronounced as in other PC loadings plots nor in the SD-C plot and the scores plot shows random noise suggesting that this was unlikely to be a real event (Figure 6.57).

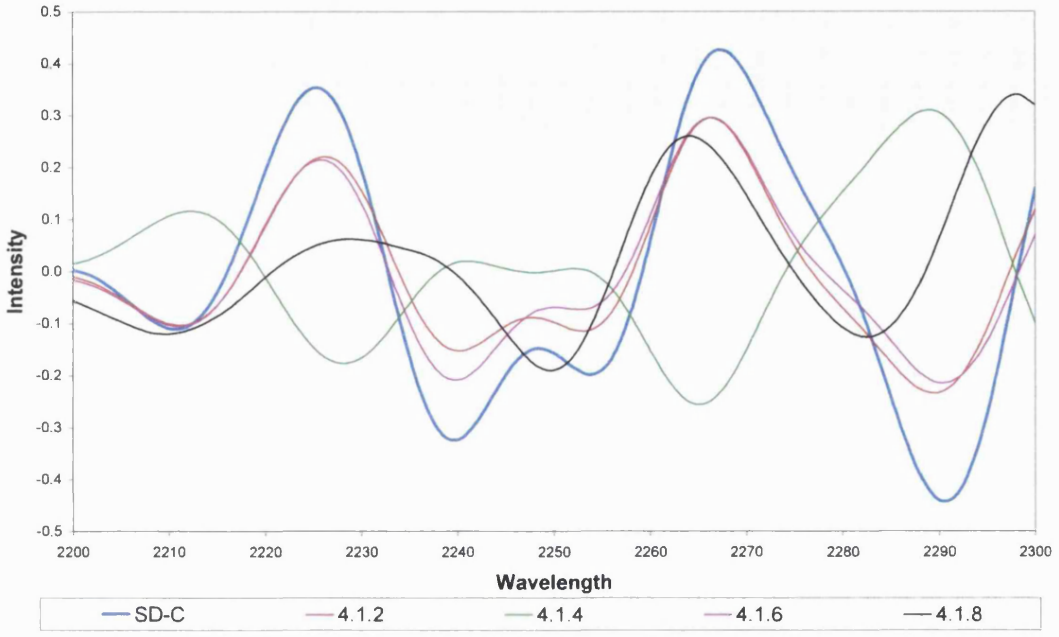

Figure 6.56 Loadings plots for each of the dispersions covering all samples stored at all conditions

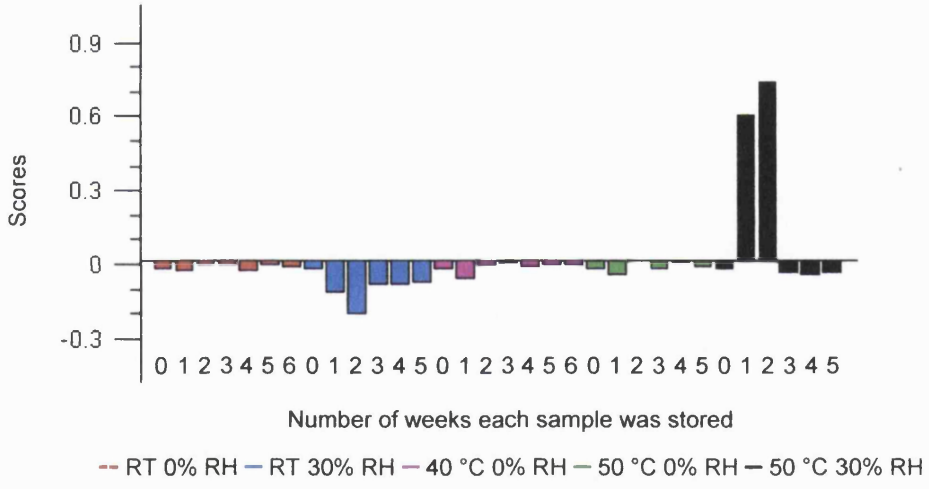

Figure 6.57 The scores plot for Dispersion 4.1.2 stored under all conditions.

The loadings plot for Dispersion 4.1.2 (binary) would suggest that it was reasonably stable at room temperature but that the higher temperatures caused griseofulvin to crystallise (Figure 6.58). In Figure 6.59, Dispersion 4.1.6 (PHPMA) was similarly shown to be stable at room temperature and also proved to be stable at 40 °C. The effect of moisture on the stability of the two dispersions was very different. The scores plot suggested that the presence of water offered a degree of protection against crystallisation in Dispersion 4.1.2 (binary), which was unexpected. Dispersion 4.1.6 (PHPMA) showed the expected relationship between water sorption and crystallisation

(at 50 °C), in that the griseofulvin crystallised more rapidly at 30% RH than at 0% RH. The XRPD data suggested that the amount of crystallisation in Dispersion 4.1.2 (binary) was broadly the same at both temperatures. Conversely there was a more obvious difference between the crystallisation of griseofulvin from Dispersion 4.1.6 (PHPMA) at the two humidities. The scores plot for Dispersion 4.1.8 which contained sucrose (not shown) did not have a significant trend.

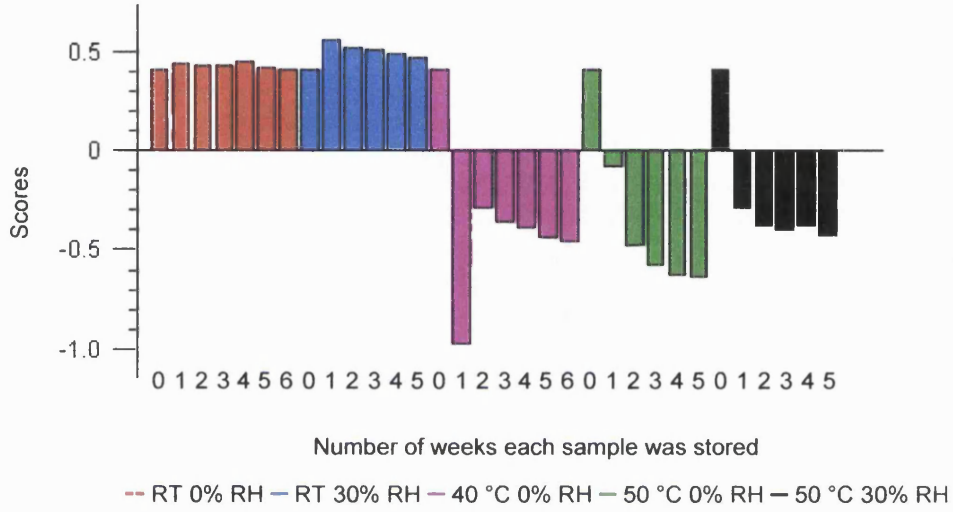

Figure 6.58 The scores plot for Dispersion 4.1.4 stored under all conditions.

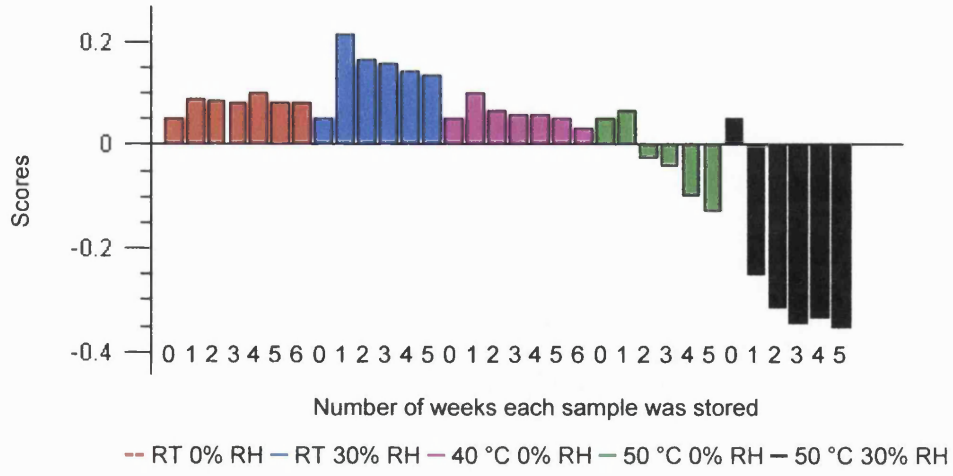

Figure 6.59 The scores plot for Dispersion 4.1.6 stored under all conditions.

The only samples of the dispersions analysed on their own were those stored at 50 °C and 30% RH, as these showed the greatest amount of crystallisation. Even here, whilst Dispersion 4.1.6 (PHPMA) displayed the PC 1 loading for crystallisation and Dispersion 4.1.2 (binary) had a PC 1 loadings plot with slightly shifted crystallisation peaks (Figure 6.60), and both had the greatest change after week 1 (Figure 6.61a and

6.59 b), Dispersion 4.1.4 (PAA) and Dispersion 4.1.8 (sucrose) did not have the characteristic crystallisation double peak. The PC 2 loadings plot for these two dispersions did not show crystallisation either (data not shown).

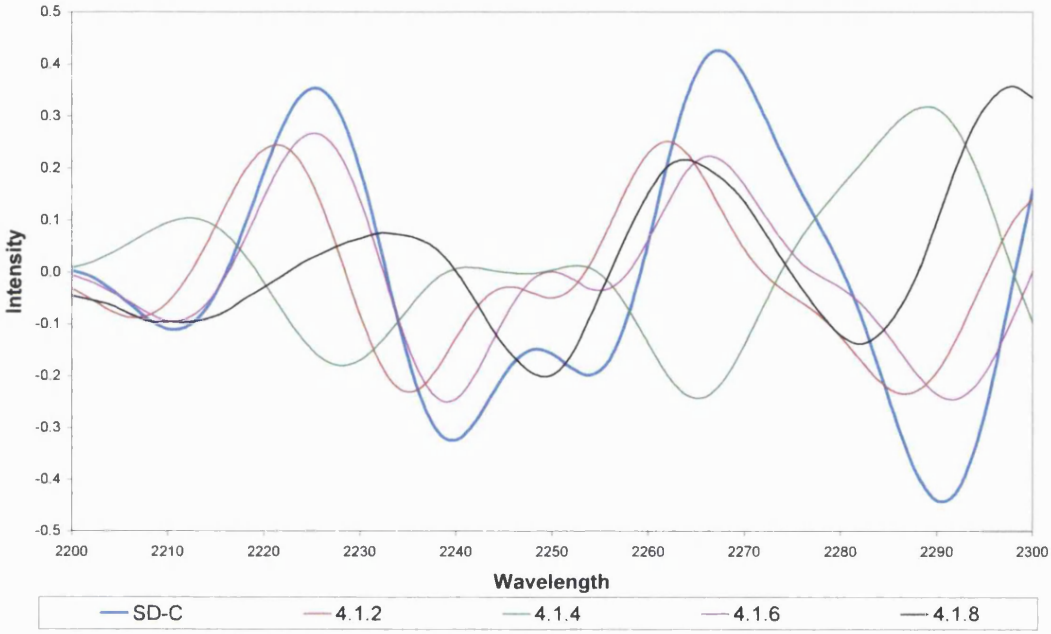

Figure 6.60 PC 1 loadings for all dispersions when stored at 50 °C & 30% RH plus PC 2 loadings for PAA and sucrose.

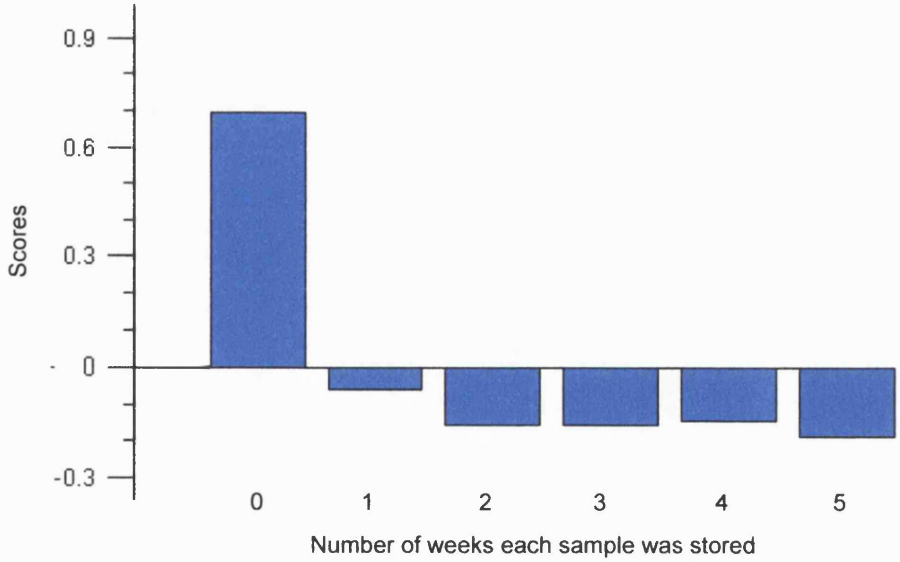

Figure 6.61a The scores plot for Dispersion 4.1.2 stored at 50 °C, 30% RH.

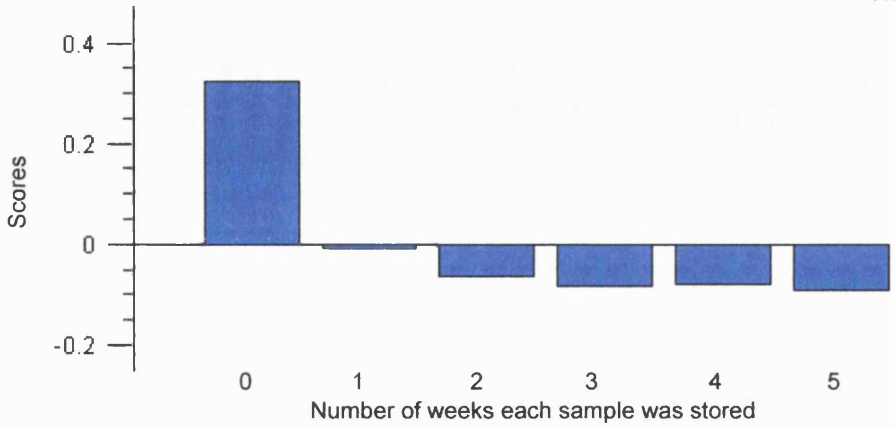

Figure 6.61b The scores plot for Dispersion 4.1.6 stored at 50 °C, 30% RH.

All the dispersions stored under all conditions have been compared together. The loadings plot shows that crystallisation double peak is the main cause of differences between the spectra (Figure 6.62). Dispersion 4.1.8 (sucrose) appears to be stable under all conditions, whilst Dispersion 4.1.6 (PHPMA) was stable under all conditions except for 50 °C, 0% RH, when it underwent a gradual change and 50 °C, 30% RH, when it underwent a more rapid change. Dispersion 4.1.4 (PAA) had a rather random scores plot and there may have been some spectral outliers although overall it would appear to be stable. Dispersion 4.1.2 showed signs of crystallisation after week 1 at 40 °C, 0% RH, 50 °C 0% RH and 50 °C 30% RH (Figure 6.63).

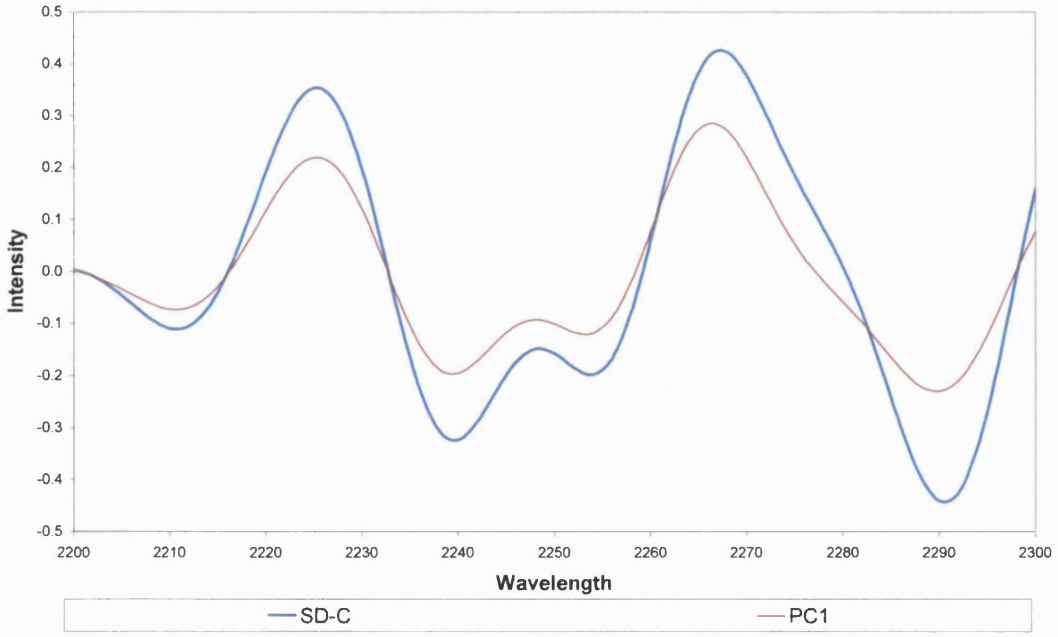

Figure 6.62 The loadings plot for all dispersion samples stored at all conditions and analysed as a single set.

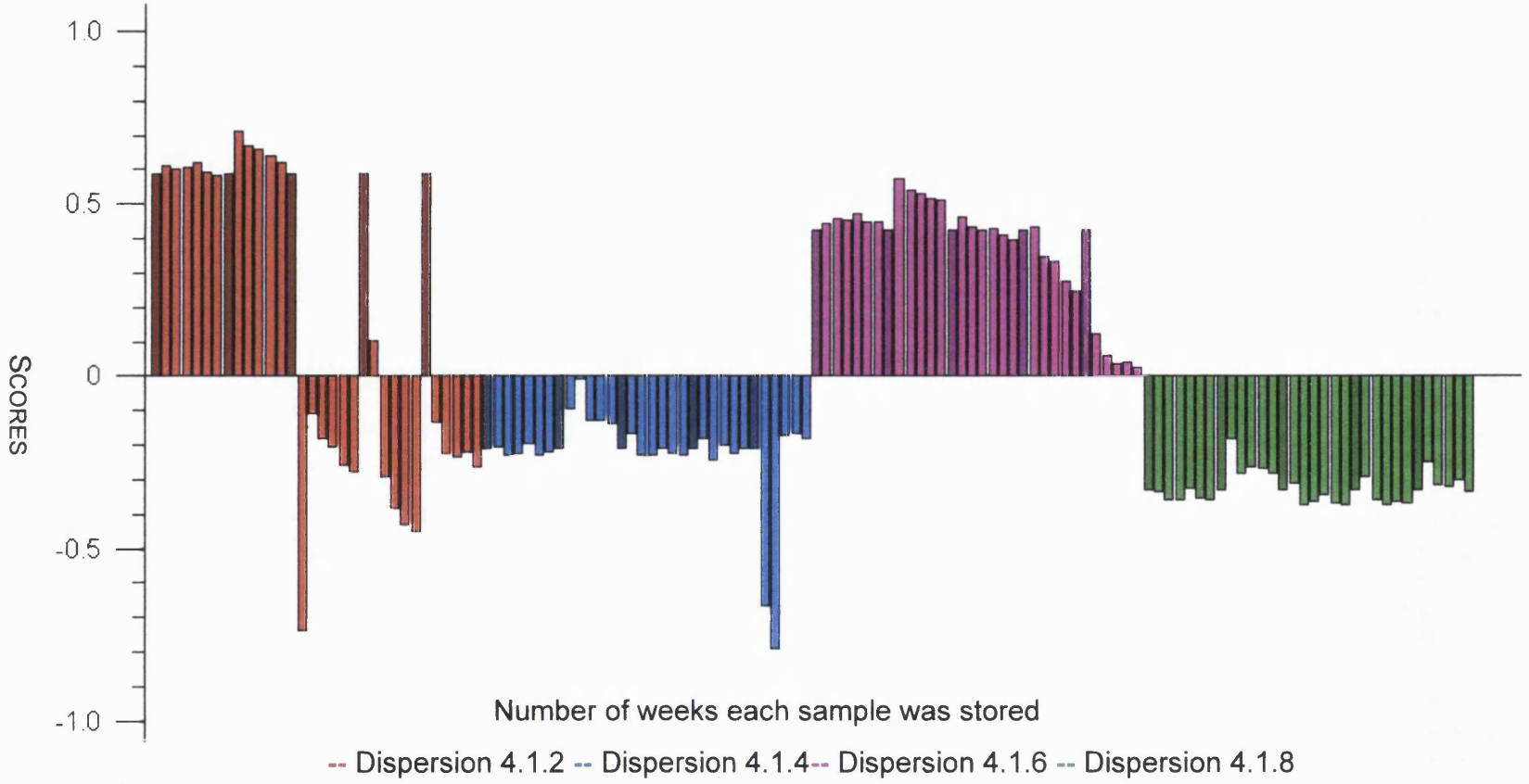

Figure 6.63 The PC-1 scores plot for all dispersions stored at all conditions analysed as a single sample set. The darker colour denotes a change in the conditions under which the dispersion was stored and these are ordered as follows; room temperature and 0% RH, room temperature and 30% RH, 40 °C and 0% RH, 50 °C and 0% and 50 °C and 30%.

6.3.9 The Changes Observed in the Water Region of Dispersions Containing Griseofulvin 40% w/w Stored at 0% RH and 30% RH

The changes in free and bound water were investigated in order to provide some information on how this relates to crystallisation. The raw spectra for Dispersion 4.1.2, which contains PVP and griseofulvin, is shown in Figure 6.64. As with Dispersion 4.1.1 (binary), changes in the spectra were observed under both 0% and 30% RH.

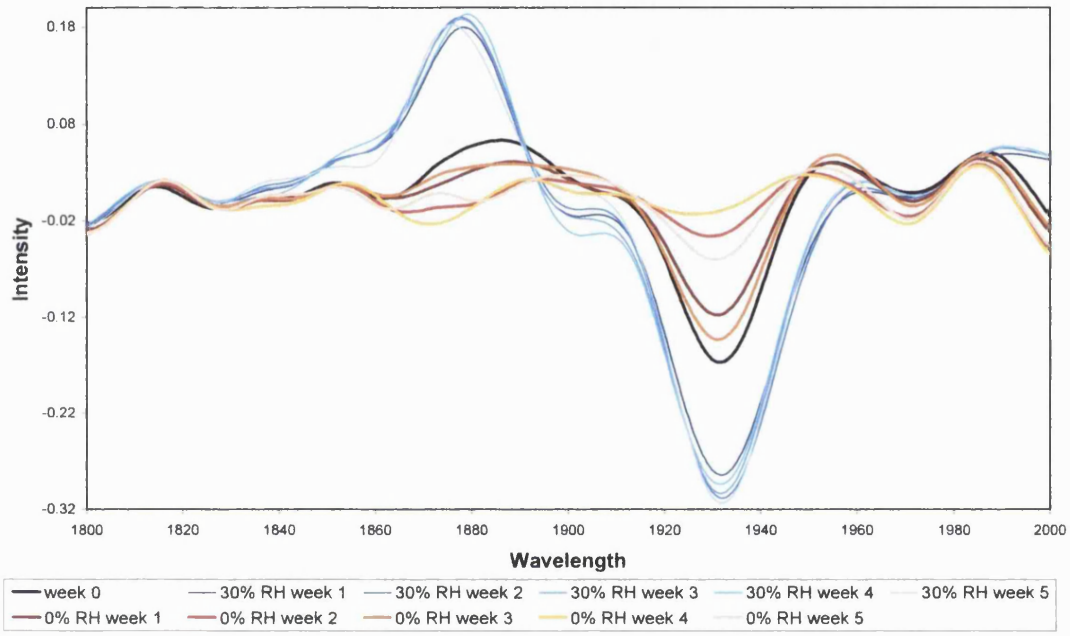

Figure 6.64 Raw spectra for Dispersion 4.1.2 stored at 50 °C and 0% RH and 50 °C and 30% RH.

The PC 1 loadings plots for each of the dispersions when stored at 50 °C, 30% RH did not display a common shape (Figure 6.65). Nor did the changes seen in these spectra match the shape of the SD-C plot. However the scores plots (data not shown) demonstrated that the majority of the changes occurred after the first week. This would suggest that PC 1 was related to the water content and subsequent hydrogen bonding changes occurring in the dispersions. The PC 1 loadings made up a high percentage of the variance between spectra (94-98%) therefore they should be considered to be valid.

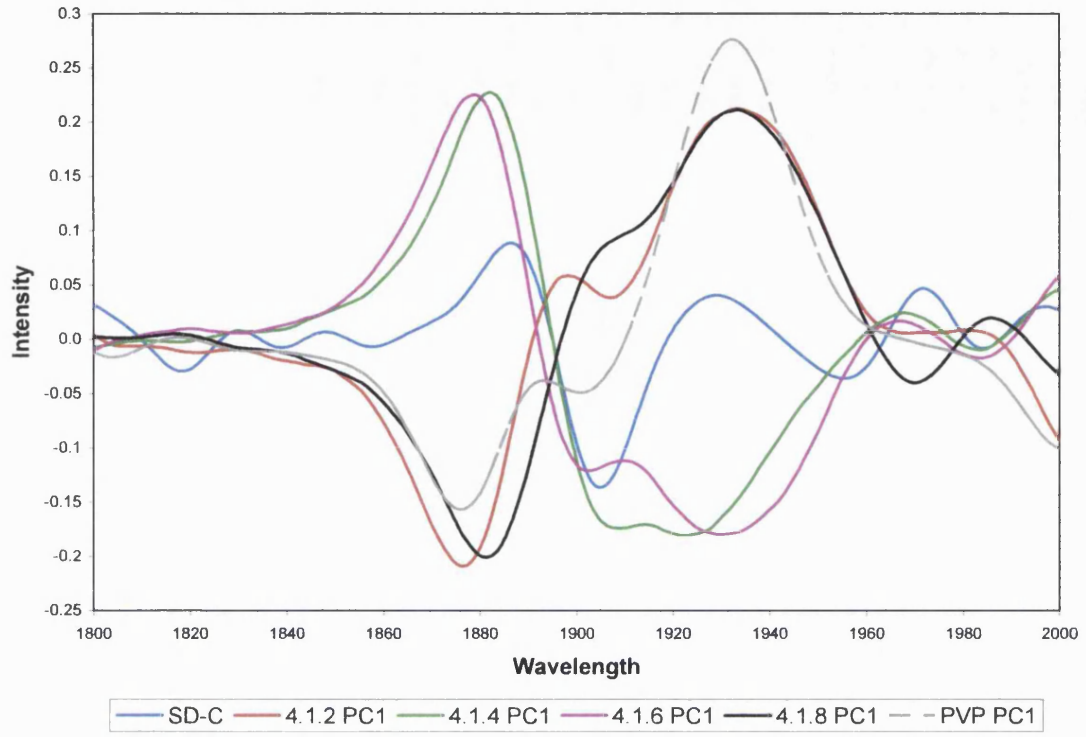

Figure 6.65 The PC 1 loadings plots for all solid dispersions containing 40% griseofulvin by weight along with the SD-C plot and the PC 1 loadings plot for PVP from the DVS-NIR.

PCA was also performed on each of the dispersions in turn, for all the other conditions (both 0% and 30% RH). The PC 1 loadings plots (data not shown) were near identical to those of the dispersions when stored at 50 °C, 30% RH (Figure 6.65). The scores plots were generally random, though the bars related to 30% RH tend to be more consistent. This again suggests that the largest change being seen between 1800-2000 nm involves bound water.

When PCA was performed on all the dispersion samples stored at 30% RH the scores plot indicated that the largest change occurred within the first week (Figure 6.66). The loadings plot was very similar to that of Dispersion 4.1.2. PCA was also performed on the NIR spectra of PVP taken during the DVS experiment and it was also found to be similar to the PC 1 plots for all dispersions. Therefore the largest cause of variance observed in these dispersions was that of water uptake and not crystallisation (Figure 6.67).

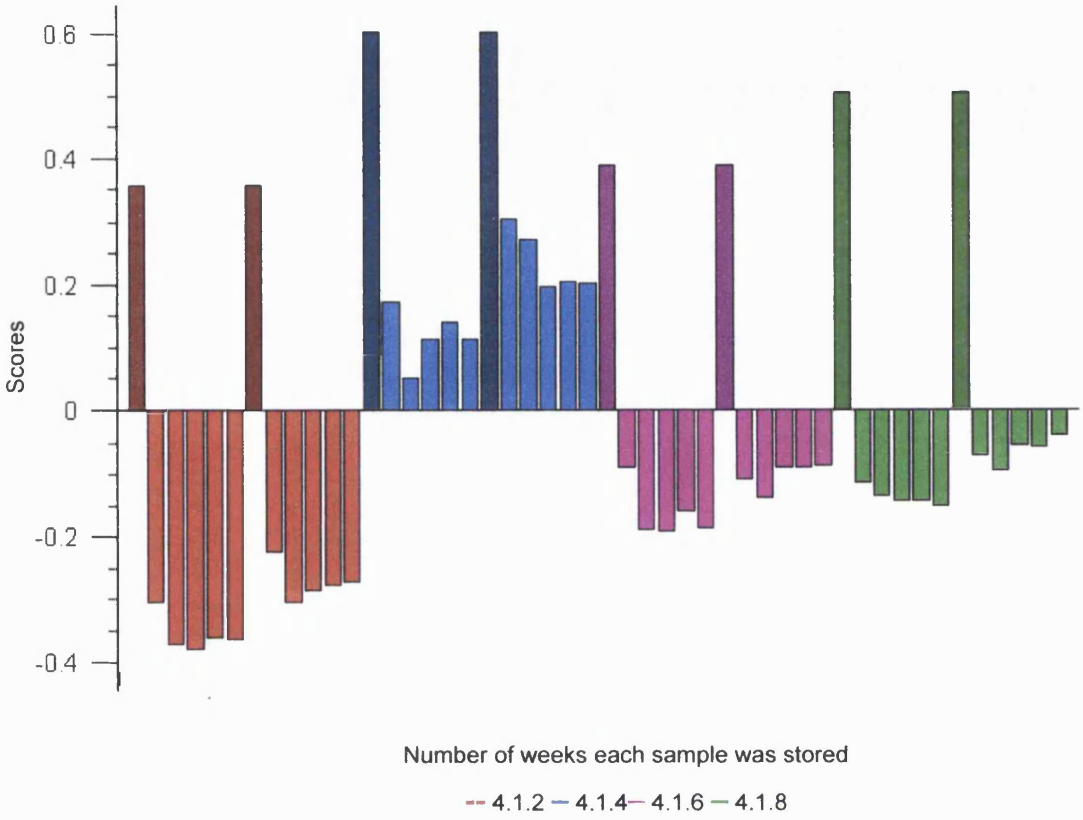

Figure 6.66 The PC 1 scores plot for all dispersions stored at 30% RH and analysed as a single sample set. The darker colour denotes a change in the conditions under which the dispersion was stored and these are ordered as follows; room temperature and 30% RH and 50 °C and 30%.

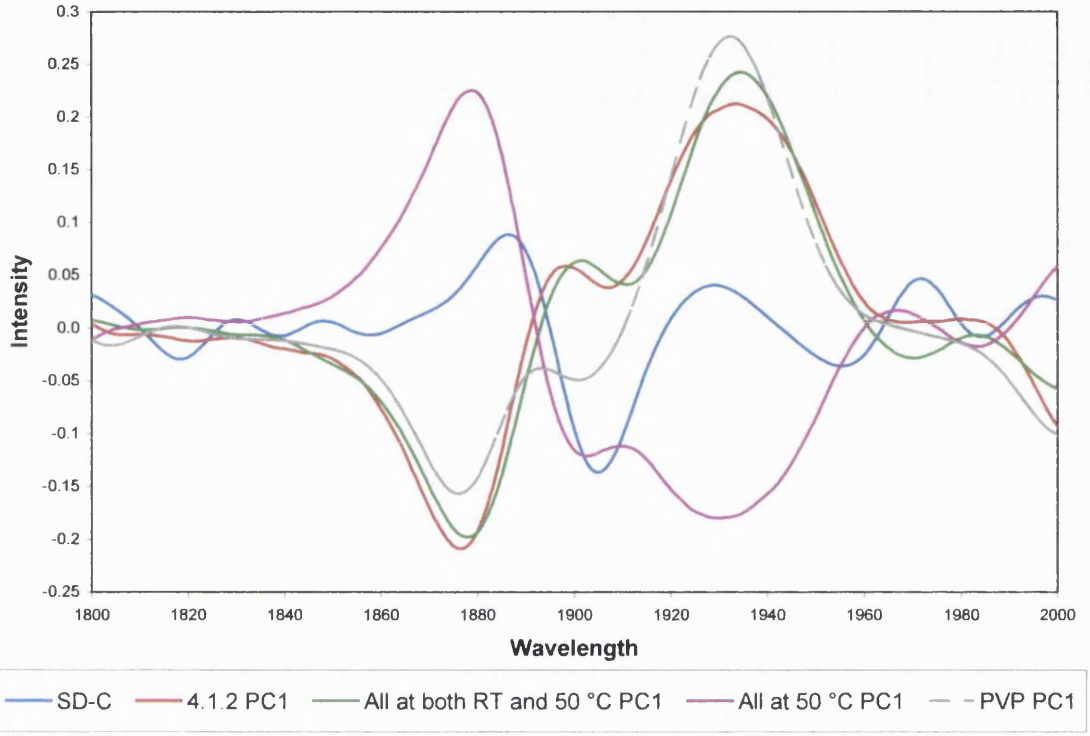

Figure 6.67 Loadings plot of all dispersions stored at 30% RH, all dispersions stored at 50 °C and 30% RH along with the SD-C plot, the loadings plot for Dispersion 4.1.2 (binary) and the PC 1 for PVP when examined in the DVS-NIR.

6.3.10 A Comparison of Dispersions Containing Griseofulvin 60% w/w and 40% w/w

Having performed PCA on all the dispersions using a variety of sets all that remained was to analyse all the dispersions stored at all the conditions as a single data set. The PC 1 and PC 2 loadings plots for all dispersions stored at all conditions are shown in Figure 6.68. The SD-C plot is also shown in Figure 6.69. This figure confirms that the largest cause of variance was that of crystallisation. The PC 1 loadings plot made up 74% of the variance within the samples whereas the PC 2 loadings plot made up 21% of the variance. The PC 2 loadings plot was similar to the PC 1 loadings plot and is therefore most likely to be due to slight peak shifts (noise) related to PC 1.

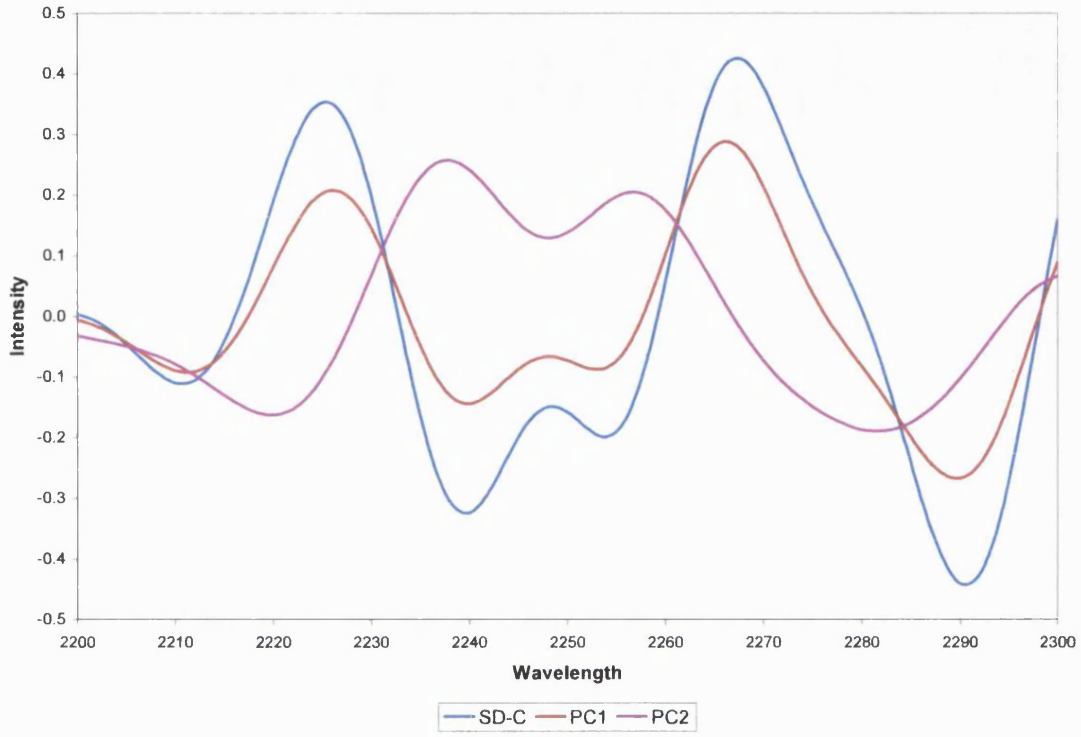

Figure 6.68 PC 1 and PC 2 loadings plots for all dispersions stored at all conditions and analysed as a single set.

The PC 1 scores plot for all the dispersions stored under all conditions and examined as a single data set is shown in Figure 6.68. Although it is quite complex it still provides some idea as to which dispersions were more susceptible crystallisation and the conditions required to induce crystallisation. Dispersion 4.1.1 (binary) was clearly unstable under most conditions. Dispersion 4.1.3 (PAA) and Dispersion 4.1.7 (sucrose) were similarly unstable at all conditions. Dispersion 4.1.2 proved to be markedly more stable at room temperature under both 0% and 30% RH however this amorphous stability would appear to have been lost at higher temperatures. Dispersion 4.1.4 (PAA) and Dispersion 4.1.8 (sucrose) would both appear to have been consistently stable across all conditions. This was not matched by the data presented in Chapter 4 in which XRPD results suggested that these dispersions were not markedly more stable than Dispersion 4.1.2. Indeed there was a tendency for both of these dispersions to be more unstable than Dispersion 4.1.2.

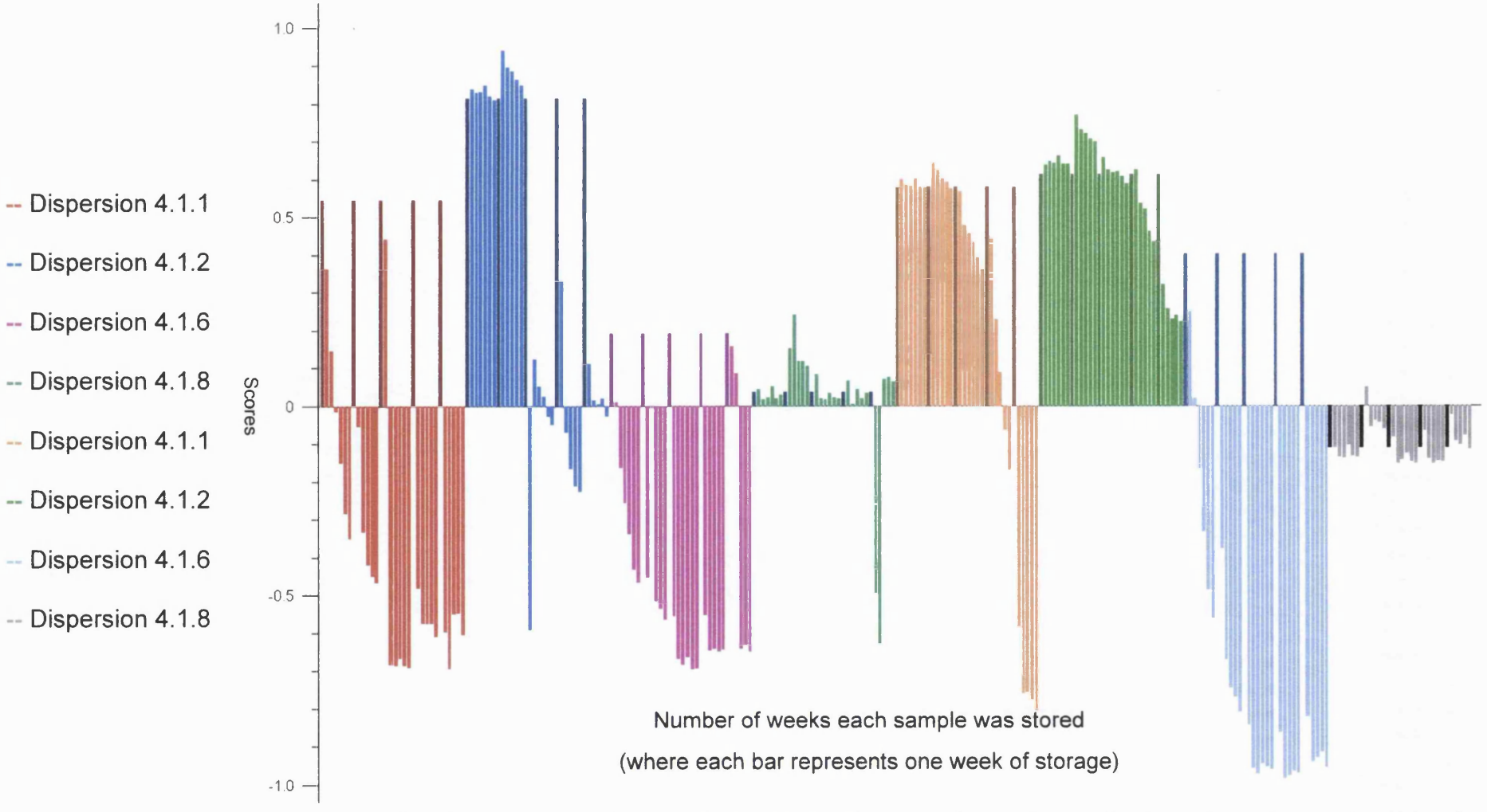

Figure 6.69 The PC 1 scores plot for all dispersions and all conditions, analysed as a single sample set. Darker colours denote changes in the conditions under which the dispersion was stored. These are ordered as follows; RT-0% RH, RT-30% RH, 40 °C-0% RH, 50 °C-0%, 50 °C and 30%.

Dispersion 4.1.5 and Dispersion 4.1.6 which contained PHPMA appeared to have been the most stable dispersions in Chapter 4. In Figure 6.69 they are again shown to have been considerably more stable than the controls; Dispersion 4.1.1 and Dispersion 4.1.2 (binary dispersions). In Dispersion 4.1.5 as in Dispersion 4.1.1 there did not appear to have been any crystallisation at room temperature irrespective of whether the samples were stored at 0% RH or 30% RH. At 40 °C there was noticeable griseofulvin crystallisation but far more crystallisation occurs at 50 °C. Dispersion 4.1.6 did not display any crystallisation below 50 °C. At 50 °C 0% RH the rate of crystallisation appears to have been consistent. At 50 °C 30% RH there did seem to have been a period of rapid crystallisation. This would appear to have been followed by a period where the amount of crystalline material remains constant. The XRPD results from Chapter 4 (Figure 4.15) showed that 23.2% of the griseofulvin had crystallised after 3 weeks whilst 45.0% had crystallised after 13 weeks. The data are not in complete agreement but both sets do suggest a more rapid phase of crystallisation.

6.3.11 CONCLUSIONS

In conclusion the NIRS does suggest that overall PHPMA is the most effective stabiliser of amorphous griseofulvin in the 60% griseofulvin samples. Of the samples containing 40% griseofulvin dispersions containing PAA (Dispersion 4.1.4) and sucrose (Dispersion 4.1.8) seemed most stable under all conditions.

NIRS results did not seem to be in complete agreement with the XRPD data. Whilst this is of concern as XRPD is considered to very effective when used to assess the level of crystallinity in a sample, with further work this limitation may be overcome. It is equally possible that NIRS shows changes that occur in a sample which are a precursor to true crystallisation. If this should prove to be the case one would have a powerful tool. There did seem to reasonable evidence that certain dispersions crystallise more rapidly than others.

It was demonstrated that water is unlikely to be a factor that directly results in crystallisation of griseofulvin from these samples and the mechanism by which griseofulvin crystallises from such high T_g systems is still unclear.

Chapter 7

Conclusions and Further Work

Many drugs are poorly water soluble and this can often limit bioavailability. One way of increasing the dissolution rate and apparent saturation solubility of a poorly water soluble drug is to prepare a solid dispersion which is a mixture of drug and excipient(s), preferably on the molecular scale. The excipient is present in order to improve the dissolution characteristics of the drug. The dissolution profile of the dispersion is often similar to that of the excipient used. The ideal solid dispersion is one in which the drug and excipient (typically a polymer) are molecularly dispersed thus stabilising the amorphous form of the drug. Such a system may be unstable and crystallisation of the drug may occur. In general such systems are more stable the pure amorphous drug though it is not yet clear how much of the stabilising effect is due to non-covalent interactions between drug and excipient and how much is due to the decrease in molecular mobility seen when a drug is dispersed in a polymer. It was hypothesised that by adding a second excipient it would be possible to stabilise an unstable dispersion. However there was a fear that this might be detrimental to the dissolution of the dispersion. The aims of this thesis were:

- To enhance the understanding of how physical properties of individual polymers affect the dissolution of drugs from dispersions containing combinations of polymers.

- To examine how blends of polymers may be used to increase the amorphous stability of poorly water soluble drugs.

- To investigate the use of near infrared spectroscopy as a method for investigating the amorphous stability of drugs in solid dispersions.

Indomethacin and polyvinylpyrrolidone (PVP) dispersions containing a variety of polyacrylates were prepared by spray-drying from organic solvent solutions and the dissolution of these dispersions were compared with that of an indomethacin and PVP dispersion. The dispersions were also characterised using differential scanning calorimetry (DSC), X-ray powder diffraction (XRPD) and thermogravimetric analysis (TGA). The dissolution studies were performed at pH 1.2, 6.5 and 7.0.

DSC and XRPD both confirmed that the spray-dried dispersions were amorphous, as was expected. When dissolution studies were performed at pH 1.2 very little

indomethacin release was seen from any of the dispersions. This was ascribed to a lack of sink conditions in the dissolution media as indomethacin is a weakly acidic drug. At pH 6.5, indomethacin dissolved from all the solid dispersions bar one containing Eudragit S. The Eudragit S dispersion only proved to be soluble above pH 7.0. A dissolution experiment at pH 6.5 covering 6 h proved that drug release would be inhibited for the entirety of the dispersion's passage through the small intestine. When the dispersion was prepared by dissolving the components of the dispersion in a different order prior to spray drying the dispersion was then found to dissolve suggesting that differences in non-covalent bonding had occurred.

Griseofulvin was spray-dried with PVP and a variety of second excipients; poly(acrylic acid) (PAA), poly(2-hydroxypropylmethacrylate) (PHPMA) and sucrose. The dispersions were then characterised by differential scanning calorimetry (DSC), thermogravimetric analysis (TGA), scanning electron microscopy (SEM), and X-ray powder diffraction (XRPD). All the dispersions were found to be amorphous with relatively similar glass transition temperatures (T_g). They contained similar levels of residual solvent and had similar morphologies. Stability studies at room temperature, 0% RH; 40 °C, 0% RH; 50 °C, 0% RH; room temperature, 30% RH and 50 °C, 30% RH were performed. The amorphous stability of these dispersions was studied by XRPD. The results showed that neither PAA nor sucrose was particularly effective in stabilising the amorphous form. PHPMA was shown to be a very effective stabiliser of amorphous griseofulvin particularly at 0% RH. A study was then performed to measure the effect that increasing the amount of PHPMA, whilst decreasing the amount of PVP, would have on the amorphous stability of griseofulvin. It was found that an increased level of PHPMA further increased the amorphous stability of griseofulvin. The order in which the components were dissolved in the feed solution prior to spray drying was also found to have an effect on the amount of griseofulvin crystallisation on storage providing a further variable for optimisation.

Flavanone, PVP and PHPMA dispersions were prepared in a similar fashion. These were also examined by XRPD in a stability study at room temperature, 0% RH. After 30 days of storage approximately 40% of the flavanone had crystallised out of these dispersions. This compared favourably with the control dispersion, Flavanone-PVP, which showed complete crystallisation after 30 days.

Clotrimazole was used as a model for the oral anti-fungals ketoconazole and itraconazole. Dispersions were prepared by spray drying the clotrimazole with PVP and a variety of second excipients. These were then placed on stability studies and characterised by DSC. The first set of clotrimazole dispersions to be investigated had a very high concentration of clotrimazole (75%) in order to rapidly assess the benefit of a second excipient. It was found that the use of excipients with the capability to donate a proton produced more stable amorphous dispersions, the excipient with the largest effect on stability proved to be sucrose. When lower concentrations of clotrimazole were used some of the benefit of sucrose was lost. The use of sucrose was found to be extremely detrimental when the dispersions were stored in humid (30% RH) conditions. PAA was found to be the most beneficial additive when the concentration of clotrimazole was at its lowest (40% w/w).

Near infrared spectroscopy (NIRS) of the amorphous stability of griseofulvin dispersions on storage suggested that the vast majority of change occurred within the first week of storage. This is in conflict with the XRPD data which suggests that crystallisation was a continuous process throughout the storage time. However NIRS did identify PHPMA as being the most effective stabiliser of amorpohous griseofulvin. It is therefore possible that the NIRS is detecting a change which is a precursor to crystallisation. If this were the case it could potentially be a powerful tool for the analysis of amorphous materials.

This work has identified a number of directions further work could follow. The mechanism by which Eudragit S prevented the dissolution of indomethacin from a PVP rich dispersion was not clearly understood. This protective effect was lost when the order in which the constituents were dissolved in the feed solution prior to spray drying was altered. Therefore spectroscopic investigations in to the differences in interactions tin these dispersions are required in order to optimise this pH-dependent release.

The work with griseofulvin, flavanone and clotrimazole has shown that the use of a combination of two polymers in a solid dispersion has some merit. In all three cases at least one of the second excipients proved to increase the amorphous stability of the drug. However, a number of components which were expected to increase amorphous stability actually proved to be detrimental. Again this effect is not clearly understood. It may be that the second excipient interacts too strongly with PVP and therefore a

degree of phase separation is "built in" to the solid dispersion. Spectroscopic investigations may help to explain this effect. The use of a combinatorial library of polymers in which the difference between each polymer is fairly consistent may also improve the understanding of these blends. Finally dissolution studies and eventually *in vivo* bioavailability studies are still required in order to demonstrate that these systems do work.

The use of principal component analysis to analyse NIR spectra in order to characterise the crystallisation of griseofulvin from solid dispersions shows merit. The system does need to be refined and the first piece of work should be to study the crystallisation of griseofulvin from a quench cooled sample stored under anhydrous conditions in order to confirm that the changes occurring in the sample are solely due to crystallisation. Should this prove to be the case one would be able to begin to develop a rapid and powerful tool for the investigation of crystallisation in amorphous samples.

Appendix One

Scanning electron micrographs of griseofulvin dispersions as described in Section 4.3.5:

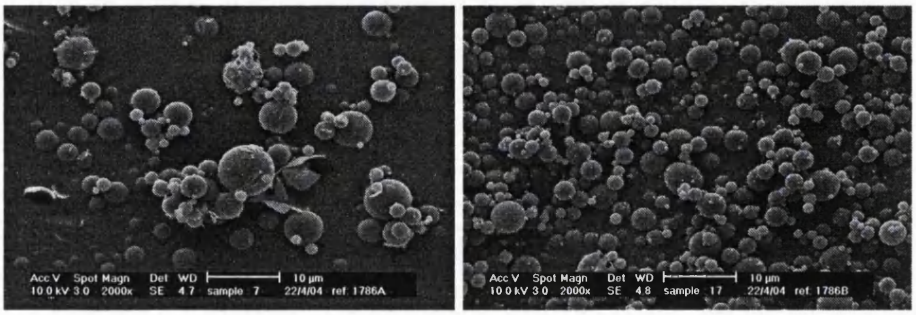

Figure A.1.1 Scanning electron microscope images of Dispersion 4.1.1 and 4.1.2.

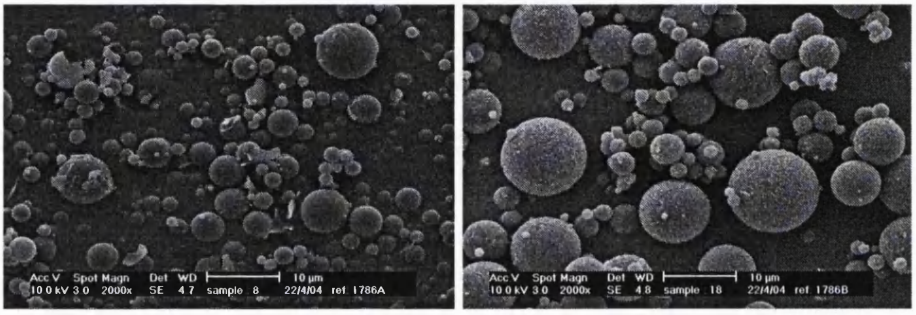

Figure A.1.2 Scanning electron microscope images of Dispersion 4.1.3 and 4.1.4.

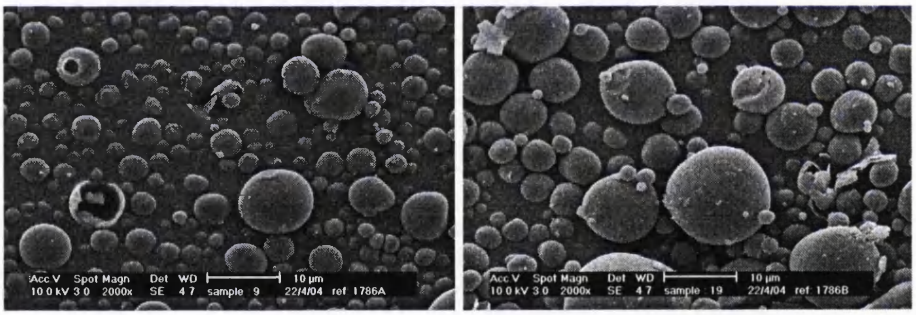

Figure A.1.3 Scanning electron microscope images of Dispersion 4.1.5 and 4.1.6.

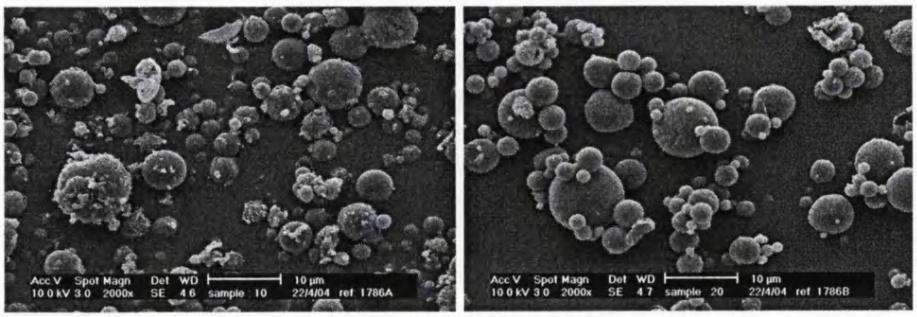

Figure A.1.4 Scanning electron microscope images of Dispersion 4.1.7 and 4.1.8.

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