Investigation into the effect of surface induced disorder on the granulation characteristics of hydrophobic actives

A thesis submitted in partial fulfilment of the requirements for the degree of

Doctor of Philosophy

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This thesis describes research conducted in the School of Pharmacy, University of London between 2003 and 2008 under the supervision of Professor Graham Buckton. I certify that the research described is original and that any parts of the work that have been conducted by collaboration are clearly indicated. I also certify that I have written all the text herein and have clearly indicated by suitable citation any part of this dissertation that has already appeared in publication.

Signature

Date 30.09.08
Abstract

The purpose of this study was to initially understand and characterise process induced transformations in hydrophobic actives and the impact upon granulation. High energy pharmaceutical processing can often lead to changes in the crystalline form and can impact upon the final formulation of the product. It is highly important that these changes in the crystallinity can be quantified and measured. Initially a technique was undertaken to characterise the amorphous content of micronised samples. The RH perfusion isothermal microcalorimeter technique was used to quantify <5% levels of disorder in the micronised active and then this technique was used to study how variables during milling such as the feed pressure and milling time can impact upon the crystallinity of these actives.

The surface transformations were also investigated using Inverse Gas Chromatography. Surface energetic data was analysed to determine the changes during processing and the changes that occur during storage of the active with an increase in the relative humidity and time. The energetic changes were studied for the two hydrophobic active to understand the impact of processing and how the active may interact during granulation.

The actives were granulated and the granule characteristics were correlated to the surface energetic data and the isothermal microcalorimetry data. The investigations yielded interesting results that indicated variability and changes in the granular distributions as a result of micronisation. Granule data such as friability of granules and the distribution of the particle sizes gave an insight into the changes that occur to the granulation system.
I dedicate this PhD to my late grandparents who came with nothing but gave me everything.
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ABBREVIATIONS AND SYMBOLS

a  Surface area of a probe
   molecule
K  Equilibrium constant
K_a  Acidic component
AN  Gutmann Acceptor Number
   (kcal mol\(^{-1}\))
K_D  Basic Component
AN*  Modified Gutmann Number
   (kcal mol\(^{-1}\))
l_n  Natural log
L  Litre
ANOVA  Analysis of Variance
API  Active Pharmaceutical
   Ingredient
m  Metre
m  Minute
MCC  Microcrystalline cellulose
BNF  British National Formulary
N  Avogado's Number = 6.02205 \times 10^{23} (mol\(^{-1}\))
BOC  British Oxygen Company
cal  Calories
K_d  Basic Component
C_t  Concentration of solute
C_s  Saturation solubility of the
   solvate
K_a  Acidic component
cos\(\theta\)  Cosine of contact angle
PVP  Polyvinylpyrrolidone
P_2O_5  Phosphorus Pentoxide
Cp  Heat capacity (JK\(^{-1}\)mol\(^{-1}\))
P/P_o  Partial pressures
D  Diffusion coefficient
P/P_o  Ratio of Inlet to Outlet Pressure
DN  Gutmann Donor Number (kcal mol\(^{-1}\))
q  Heat (J)
R^2  Correlation coefficient
R  Gas constant = 8.314 (JK\(^{-1}\)mol\(^{-1}\))
DSC  Differential Scanning
   Calorimetry
F  Flow rate (mL min\(^{-1}\))
F  Force
FID  Flame Ionisation Detector
S.D.  Standard deviation
g  Gram
SMS  Surface Measurement Systems
G  Gibb's Free Energy For
   Sorption (kJ mol\(^{-1}\))
t  Time (s)
GMP  Good Manufacturing Practice
   t_r  Retention time
h  Hour
   t_0  Reference elution time
H  Enthalpy (kJ mol\(^{-1}\))
   t_n  Net retention time
HDSC  Hyper Differential Scanning
   Calorimetry (HyperDSC)
IUPAC  International Union of Pure and
   Applied Chemistry
I  Joule
K  Kelvin
   UV  Ultra Violet
   V  Volume
TCD  Thermal Conductivity Detector
TAM  Thermal Activity Monitor
CHAPTER ONE

INTRODUCTION
1.1 CRYSTALLINE STATE

An ideal crystalline material is defined as a structure with a unique arrangement of molecules repeated periodically in three dimensions. The molecules are arranged in a repeating set along points of a lattice, these repeating sets are termed as the unit cell regularly repeated in a three-dimensional space. The unit cell has a definite orientation and shape defined by the translational vectors, a, b and c with a definite volume, V. An example is shown in Figure 1.1

![Figure 1.1 - Schematic diagram of an ideal crystalline structure NaCl](image)

The molecules and atoms are contained within the lattice structure necessary for generating the crystal. The lengths of the unit cells and the angles between them are known as lattice parameters and the space group defines the symmetry that is intrinsic within the structure. The structure of a given crystal can be assigned to one of 14 Bravais lattices and to one of the 230 space groups (Brittain and Byrn, 1999). The structure of a crystal and the symmetry determines many of the physicochemical properties of a material. Many pharmaceutical drugs and additives exist as crystalline materials or have a high degree of crystallinity. The crystallinity of pharmaceutical products is very important during formulation as changes to the crystal form can have an
adverse effect on the final product characteristics. Therefore it is highly important to understand these changes and understand the processes attributed to them.

1.2 CRYSTALLISATION

Crystallisation is the formation of solid crystalline material from a homogenous solution. Under specific conditions such as supersaturation of the solution and temperature, crystallisation may proceed as two distinct phases, nucleation and crystal growth. Primary nucleation is the first step in crystallisation, this is where solute molecules that are dispersed within the solvent gather into clusters. Under specific conditions these clusters reach a critical size to form stable nuclei where a defined and periodic arrangement defines the crystal structure. Crystal growth is the continuous growth of the nuclei from the nucleating site that can occur very rapidly in comparison to nucleation. The process is complete once the supersaturated solution reaches equilibrium.

1.3 POLYMORPHS

Crystalline materials may exist as more than one distinct crystal species and are termed polymorphs. Polymorphs have the same chemical composition but differ in the crystal arrangement within the lattice structure. The different crystal structure in polymorphs arises when the substance crystallises with a different packing arrangement. Due to the different crystal structures polymorphs will have differing lattice energies. This will result in physical properties such as melting point, density, hardness, enthalpy of fusion and dissolution rate to differ across polymorphs. An example of this is carbon that can exist as the cubic form (diamond) or hexagonal (graphite) form with distinctly different properties. The most stable polymorph will have the highest melting point however the lowest rate of dissolution. Pseudopolymorphs are crystalline solids with solvent molecules embedded within the crystalline structure, in either stoichiometric or nonstoichiometric proportions. They are also known as solvates and if the solvent is
water it is known as a hydrate. This can often lead to many changes in the physicochemical properties of the active (Vippangunta and Brittain, 2001).

1.4 AMORPHOUS STATE

Within a crystalline material there exist imperfections, defects or regions of complete disorder. If this disorder is extensive throughout the structure of the crystal it is termed as an amorphous structure. This disorder can exist within a crystal or may be unintentionally induced through the use of high energy processes. The amorphous form is a thermodynamically unstable form with greater molecular motions and a higher internal energy and volume compared to the crystalline form. The amorphous structure consists of particles with a more random orientation and no distinct three-dimensional long-range order that exists in a crystalline material. Short-range order exists between particles over a few molecular dimensions within the structure and the molecules are more fluid similar to the liquid state. Figure 1.2 displays a representation of the arrangement of the molecules in the various states.

![Schematic representation of molecular arrangement](image)

Figure 1.2 – Schematic representation representing molecular arrangement of amorphous and crystalline material (Yu, 2001)
Due to the changes in the molecular arrangement of the structure there will be inherent differences to the physicochemical properties of a material when in the amorphous state as compared to its relative crystalline state. The differences are discussed in Section 1.8 however it is important to understand how amorphous material is formed to understand the molecular changes that occur.

1.5 GLASS TRANSITION TEMPERATURE

The glass transition is an event within a amorphous system that is dependent on a materials properties and related to the time, temperature and enthalpy of the system where the molecular mobility causes a change from a glassy amorphous material to a rubbery liquid amorphous state as the temperature is below and above the $T_g$ respectively.

The $T_g$ is associated with a change in the material heat capacity without an associated heat transfer such as that during crystallisation. The $T_g$ describes a transition that results in a change in the material properties, as the temperature is increased the material properties change from a brittle material more rigid in structure to a more fluid rubbery state. This can have consequences in formulation and the measurement of the $T_g$ can be an important factor if a material may undergo changes in crystal form during development.

1.5.1 The glassy state theory

As a material is cooled from the melt there is a change in the volume and enthalpy, this change will depend upon the rate at which the material is cooled. A schematic diagram of a change in volume or enthalpy with temperature for a material is shown below in Figure 1.3. If the material is cooled slowly the liquid begins to undergo structural changes as the mobility of the molecules decrease. The molecules have sufficient time to undergo crystallisation as there is a significant decrease in the volume and enthalpy. The
molecules arrange into crystal lattices as the arrangement decreases in volume occupied. As the material is cooled further there is a very slow rate of volume reduction. If the material is re-heated it will have the same melting temperature ($T_m$).

A supercooled material has a higher free energy and the molecules within the material do not have sufficient time to arrange into a crystalline lattice. The molecules remain in a more randomly orientated arrangement as the mobility of the molecules is decreased. The material undergoes a slower rate of volume and enthalpy change as the material cools at a temperature below the crystallisation temperature. The Kauzmann paradox states that if the temperature decrease is sufficiently rapid, the contraction curve would continue across the curve corresponding to the solid ($T_K$ – Kauzmann temperature). $T_K$ is the temperature at which the entropy of the liquid becomes the same as the crystalline material. In reality this process does not occur and the curve deviates due to the glass transition temperature.

![Figure 1.3 - Schematic of the variation of volume and state transformation related to temperature changes (Hancock and Zografi, 1997)](image-url)
At the glass transition temperature there is a sudden and rapid decrease in the molecular mobility due to the viscosity of the system. As mentioned previously, below the $T_g$ the material has a more brittle nature and above the $T_g$ is similar to that of a material in the rubbery state.

The $T_g$ can be calculated experimentally using a variety of techniques including calorimetric, thermomechanical, volumetric and some spectroscopic methods. The method most commonly used to calculate the $T_g$ is Differential Scanning Calorimetry (Chapter 2.3) where the heat capacity change is recorded typically as an endothermic baseline shift. The $T_g$ can differ under certain circumstances, the cooling rate being one, where a slower cooling rate can lead to a lower $T_g$. Another example is the inclusion of compatible additives that can either have a lowering effect on the $T_g$, termed plasticisers or an increase in $T_g$ termed anti-plasticisers.

1.6 PLASTICISER

A plasticiser is a molecule that dissolves within a solid structure and increases the free volume of the material thereby increasing the opportunity for molecular mobility and decreasing the glass transition temperature (Hancock and Zografi, 1994).

Many compounds can have a plasticising effect such as phthalate used in the plastic manufacturing industry, alkyl citrate used in the food industry and benzoates to name a few. Water is a molecule that can have a plasticising effect due to its small molecular size and the ability to disrupt hydrogen bonding within a material. This makes it an effective plasticiser and once incorporated in an amorphous material can often reduce the $T_g$ markedly (Ahlneck and Zografi, 1990). Since there is a mixture of two components one being the active and the other the plasticising compound a two component model on the change in the $T_g$ can be approximated.
1. INTRODUCTION

The Gordon Taylor equation (Gordon and Taylor, 1952) describes the estimation of the $T_g$ whereby two components have been mixed and based on the free volume theory. For a two component mixture the equation is as follows:

\[
T_{g\text{mix}} = \frac{w_1T_{g1} + kw_2T_{g2}}{W_1 + kw}
\]

Where:

- $T_{g\text{mix}}$ = $T_g$ of a mixture containing components 1 and 2
- $T_{g1}$ = $T_g$ of compound 1
- $T_{g2}$ = $T_g$ of compound 2
- $w_1$ = weight fraction of component 1
- $w_2$ = weight fraction of component 2
- $K$ = constant which is a measure of the free volume contributed to the mixture by each of its components at any temperature where $k$,

\[
K = \frac{\rho_1T_{g1}}{\rho_2T_{g2}}
\]

where:

- $\rho_1$ = density of component 1
- $\rho_2$ = density of component 2

The equation can be used to estimate the $T_g$ of a compound and the effect a plasticiser may have on the system. An example of a potent plasticiser encountered is water with a $T_g$ of 135K and is absorbed readily by many pharmaceutical additives. The use of Equation 1 can predict the influence a certain level of water introduced within a system may have on the $T_g$ of the amorphous material. Considering storage conditions of many...
pharmaceutical actives this can be a useful tool in predicting performance where amorphous material may be present. Due to the significant differences between the crystalline and amorphous state it is highly important to understand the Tg of a material prior to use.

1.7 AMORPHOUS TO CRYSTALLINE TRANSITION

The amorphous state is in an unstable thermodynamic state and will revert to the crystalline state once the Tg is either lowered to that of operating temperatures or if the temperature is raised above Tg. As noted previously the incorporation of plasticisers can lower the Tg sufficiently to initiate the crystallisation process. It has been shown (Hancock et al., 1995) that the temperature must be kept 50K below the experimental Tg to prevent crystallisation. As the temperature is increased above the Tg there is an increase in molecular motions and a change in the viscosity and volume within the structure. A process known as collapse is observed where the structure may subside depending on experimental and material properties. During the crystallisation process water is expelled as the free volume decreases. Some water may remain attached to the structure and the material may form a hydrate.

1.8 DIFFERENCES BETWEEN THE CRYSTALLINE AND AMORPHOUS STATE

Due to the difference in the molecular arrangement compared to the relative crystalline state physicochemical properties differ. Since there is a more random arrangement of particles and the molecules are further spaced from one another the specific volume is greater and the density is lower than that of a crystal. The presence of amorphous regions can be beneficial in terms of enhanced dissolution rate and increased bioavailability (Chiou and Kyle, 1979) yet many of the property changes such as instability and change in processing properties (Sebhatu et al., 1994a) are undesirable. Amorphous materials are thermodynamically unstable and will tend to revert to the
crystalline form under certain conditions of temperature, humidity and pressure. Amorphous material can be induced within a crystalline material through a variety of processes that can cause a significant amount to be present on the surface of the material. This disorder is also known as 'reactive hot-spots' and are primarily located on the surface of the material. Mechanical activation such as milling, spray drying, granulation and mixing can all lead to changes in the crystal structure that may go unnoticed during manufacturing.

1.9 PROCESS INDUCED DISORDER

As mentioned earlier many pharmaceutical processes can unintentionally render a crystalline material amorphous or partially amorphous. Techniques that exist now are able to identify this level of disorder which may only contribute a minor proportion of the bulk however as this disorder is primarily on the surface constitutes a large percentage that will affect surface mediated reactions.

1.10 MILLING (COMMINUTION)

Drug compounds are often milled so that a defined smaller particle size distribution is available and mainly to improve dissolution properties of poorly soluble drug compounds. Micronisation can be employed to reduce particles to a size range of 10μm or less and can greatly improve dissolution profiles of compounds. Unfortunately, there are many disadvantages associated with the milling of compounds including surface changes, poor flow properties and the increase in toxicity due to dust formation. The actual process of milling is also extremely inefficient (Parrott et al., 1990).

The high-energy process often results in a decrease in crystallinity due to the formation of lattice defects located primarily at the surface. The defects created by mechanical activation can migrate, transform and change their number and nature (Vippagunta et al., 2001). If the defects created by the mechanical activation produce a lattice that is
different from the initial lattice a polymorphic transformation takes place. Polymorphic change created by milling has been noted for many drug compounds such as fostedil, chloramphenicol palmitate, indomethacin and phenylbutazone. As mentioned previously polymorphic and amorphous forms of the drug must be determined to prevent batch-to-batch variability. It is important to recognise the type of milling equipment used and the operating technique by which it reduces particle size. Various milling techniques exist including hammer mills, vibration ball mill and air jet mills.

Following administration the dosage form should release the drug into solution at the optimum rate. The rate of solution depends on many factors, one of which is particle size. The particle size of a compound can therefore greatly influence the production of a dosage form. A change in particle size has a combination of effects that may result in a change of the physicochemical characteristics. Reduction in size generally increases the specific surface area of particles. According to the Noyes-Whitney equation (Equation 3) an increase in the specific surface area will tend to increase the rate of solution.

Rate of solution of solids when the process is diffusion-controlled and involves no chemical reaction.

Equation 3  \[ \frac{dc}{dt} = \frac{D}{Vh} \cdot Sc \cdot (C_s - C_t) \]

where:

D is the diffusion coefficient,

h is the thickness of an assumed stagnant diffusion boundary layer,

V is the volume of the solvent,

C_t the concentration of the solute

C_s is the saturation solubility of the solvate in bulk

dw/dt = rate of increase of amount of material in solution dissolving from a solid.

S_c is the effective surface area for dissolution
The Noyes-Whitney equation demonstrates that solubility is one of the main factors determining the rate of solution. The maximum equilibrium solubility of a drug in a given medium is highly important as it dictates the rate of solution of the drug. The higher the solubility the more rapid is the rate of the solution. Micronisation reduces the particle size of the compound and increases the surface to volume ratio. This increases the surface area exposed to solvent and leads to an increase in the rate of solution (\(dw/dt\)). Many techniques exist for the reduction in particle size, but largely many pharmaceutical products are still mechanically reduced with the use of processing equipment. Two examples used in this study were the fluid energy mill and the vibration ball mill.

1.10.1 Ball milling

Ball milling involves the use of a grinding medium such as porcelain or steel balls to cause breakage and attrition using a mechanical force within either a rotating or vibrating vessel. Ball mills grind by the force of impact and attrition. The vibration ball mill method is a very efficient process in comparison to other methods and size reduction to micron size is achievable. When the mill is fully operational the load nearest to the wall of the cylinder breaks free and is quickly followed by other sections so that top layers of balls travel at a faster speed than the lower layers that causes attrition of material between them. The quantity of grinding medium is expressed in terms of percent volume of the mill. For optimum results the mill should be filled with 50% grinding medium (Parrott, 1974). The amount of material to be milled should fill the void and just cover the grinding medium. If greater than 25% of the total volume of the mill is occupied by material the balls will not contact sufficiently to cause breakage (Parrott, 1974). Smaller sized grinding medium facilitate fine grinding as they provide smaller voids than larger balls; consequently the void through which the material can flow without being struck by a ball is reduced and number of impacts per unit weight of material is greater. Hence, the smallest sized grinding medium that fragments particles is recommended. The main factors that are important in a ball mill will be the occupied space within the vessel, number of milling balls, speed of rotation or vibration, amount
of material to be milled and the duration.

1.10.2 Fluid energy milling

Fluid jet mills achieve micron-sized particles and have a number of advantages compared to standard mechanical reduction methods. Size reduction typically achieves a narrow particle size distribution, the method is suitable for heat-sensitive compounds and the process has a low risk of contamination. Many different types of fluid jet mills exist such as spiral or loop jet mills, impact and counter flow jet mills. A schematic of a typical spiral jet mill is displayed in Figure 4. They all operate with the same principle with the use of fluid energy to achieve grinding by particle impact and attrition. The amount of energy to cause fracture depends on the hardness and particle size of the compound and the type of stress applied. The fracture theory states that the hardness of a material increases as its particle size decreases. Therefore the milling of finer particles requires a greater amount of energy in order for fracture to occur. As particles are reduced in size impact speeds are likely to be reduced due to an increase in air friction, greater surface area and interparticulate collisions more likely to occur.

Figure 1.4 - Schematic diagram of a spiral jet mill (Midoux et al. 1999)
1. INTRODUCTION

The material is fed through a hopper and the feed rate may be adjusted. The injector gas accelerates the product through the mill chamber and a cyclone develops that produces a centrifugal force. The gas accelerates in the opposite direction and the particles within the stream to impact with other particles in the mill chamber. Once the particles attain a sufficient particle size they will enter through the particle outlet as the centrifugal force allows the passage of finer particles. The particles are then deposited in the collecting chamber at the bottom of the device and the gas escapes through the top half which consists of a mill bag. The mill bag collects finer particles that are suspended in the gas flow. The superfine particle in the mill bag can account for 15-20% of the end product and so the two fractions are combined.

Spiral air-jet mills utilise particle impact and attrition to induce particle size reduction, with the ability to produce micron sized particles between 1-15μm (Midoux et al, 1999). The degree of comminution depends on the critical milling parameters. The most important parameters are feed rate, feed pressure and grind pressure. Depending on the level of energy input milling can give rise to disorder, defects or even amorphous regions on the surfaces of crystalline material. Even small quantities of amorphous product can have a marked effect on the physical and chemical stability of a product.

1.11 SURFACE ENERGETICS

It has been shown how a high energy process such as milling can change the properties of a crystalline material and render it partially amorphous. This is critical in pharmaceutical applications where many processes are dependent on the surface properties of the material. The intermolecular forces responsible for the surface properties can be described using the surface energy. The surface energy (\(\gamma\)) consists of a number of different physical forces and defined as the energy necessary to create a unit area of surface. The two components that combine to describe the surface energy are known as the non-polar or dispersive (\(\gamma^D\)) and polar (\(\gamma^P\)) components.
1. INTRODUCTION

At the surface an interfacial energy will be created that is dependent on the polar and non-polar interactions. The individual properties of the two components will govern the surface-mediated reactions. The extent of the interfacial free energy will dictate many processes such as wetting (Krycer et al, 1983), binding, granulation (Rowe, 1989) and mixing. It is important to understand the influence the surface energy will have on a process as changes will influence many pharmaceutical processes. The measurement of the surface energy can be undertaken by two main techniques, the contact angle method and inverse gas phase chromatography.

1.12 CONTACT ANGLE

The contact angle method is the technique whereby a liquid droplet is used to determine the interfacial forces of the material. The shape of the angle formed between the surface and the liquid is known as the contact angle and this is used to calculate the surface energy. Liquids that have a low contact angle (< 90°) will spread across the surface indicating greater wettability and liquids that have a higher contact angle (> 90°), notably forming a droplet at the surface will indicate poor wettability. The contact angle is formed at the solid/liquid/vapour interface as a static drop on the surface, however there is a range of angles as the drop interacts with the surface. When the drop has expanded the liquid represents the ‘advanced’ contact angle and when the liquid contracts the angle is said to represent the ‘receded’ contact angle. The angles fall within a range of advanced angles and receded angles and is described as the dynamic contact angle.
1. INTRODUCTION

There are a variety of different techniques to measure the contact angle, two techniques that are used are goniometry and tensiometry. Goniometry involves the use of a sessile drop of test liquid on a solid substrate. Tensiometry involves the use of solids and the forces when in contact with the test liquid. If the forces of the interaction, the geometry of the solid and the surface tension of the liquid are known the contact angle may be calculated using the equation below:

\[ F = \gamma_{LV} \cos \theta \]

Where:

- \( F \) is the force
- \( \gamma_{LV} \) is the surface tension of the liquid/vapour interface
- \( \cos \theta \) is the cosine of the contact angle.

The surface tension of the measuring liquid must be accurately measured prior to the test. To measure the surface energy the dimensions of the solid and three different test liquids with their respective surface tensions must be known. Two of the liquid must be polar and one must be non-polar. In the example of the Wilhelmy plate technique, the solid can be evenly distributed to a glass slide and the resulting force measured. From the results in the various liquids the surface energy components can be calculated (Rillosi and Buckton, 1995).
The primary aim is to determine the wetting characteristics of the solid/liquid interaction. There are a number of parameters that can be used to describe the material properties.

The work of adhesion is defined as the work required to separate the solid and liquid also known as the free energy associated with the adhesion of the solid and liquid phase. This parameter gives an indication of the strength of the interaction between the two phases. It can be given by the Young-Dupre equation:

Equation 6 \[ W_a = \gamma (1 + \cos\theta) \]

The work of cohesion is the energy required to separate the material into two parts and is a measure of the strength of the molecular interactions in the material. It is defined as:

Equation 7 \[ W_c = 2\gamma \]

The work of spreading is the negative free energy associated with the spreading liquid over the solid surface. This is also known as the spreading coefficient and is given by:

Equation 8 \[ W_s = \gamma (\cos\theta - 1) \]

The wetting tension represents the cosine of the contact angle and allows for the characterisation of the strength of the wetting interaction without a separate measurement of the surface tension. This may be itself in situations where the surface tension at the interface may not be equal to the equilibrium surface tension.

Equation 9 \[ \tau = F_w / P = \gamma LV \cos\theta \]
1.13 CHARACTERISATION OF THE SURFACE

Measurements of the surface tension are an indication of the thermodynamic properties of the materials investigated. The measurement of the contact angle yields data that reflects the wetting behaviour of the particular solid/liquid pair. Various methods are used to calculate the critical surface tension and the surface free energy.

1.13.1 Critical surface tension

The critical surface tension is an indication of whether wetting will be favourable. A low critical tension represents a low energy per unit area. Using a series of homologous liquids of differing surface tensions a graph of $\cos \theta$ vs $\gamma$ is produced. The data will form a line which approaches $\cos \theta = 1$ at any given value of $\gamma$. This is the maximal surface tension of a liquid that may wet the solid material.

1.13.2 Surface free energy

Another way to characterise the solid surface is to calculate the free surface energy. This approach involves testing the contact angle against a series of liquid, two being polar and one non-polar. The values for the polar and dispersive components and the surface tension must be known. The equation used by Owens and Wendt as:

Equation 10

$$\gamma_i \frac{(1 + \cos \theta)}{(\gamma_{id})^{1/2}} = (\gamma_{sp})^{1/2} \left[ (\gamma_{lp})^{1/2}/(\gamma_{id})^{1/2} \right] + (\gamma_{sd})^{1/2}$$

where $\theta$ is the contact angle, $\gamma_i$ is liquid surface tension and $\gamma_s$ is the solid surface tension, or free energy. The addition of $d$ and $p$ in the subscripts refer to the dispersive and polar components of each. The form of the equation is of the type $y = mx + b$. A graph of $(\gamma_{lp})^{1/2}/(\gamma_{id})^{1/2}$ vs $\gamma_i \frac{(1 + \cos \theta)}{(\gamma_{id})^{1/2}}$ can be constructed and the slope will
be \((\gamma_{sp})^{1/2}\) and the y-intercept will be \((\gamma_{sl})^{1/2}\). The total free surface energy is the sum of
the two component forces.

1.14 DISADVANTAGES OF CONTACT ANGLE METHOD

Although the contact angle method is useful at determining the surface energy of a
material there are many disadvantages. The primary issue is the surface heterogeneity of
materials under investigation. Surface roughness can cause variation in the contact angle
noted by a hysteresis during measurement using this technique. Many values can be
obtained between the maximum advancing angle and the minimum receding angle.
Techniques exist to limit the effect of surface roughness however they are often
unsuitable due to changes that occur to the surface energy during handling. Examples
include compaction of the material and the use of adhesives for powdered samples. The
technique used to prepare the sample can also have a bearing on the contact angle
measured. It has been shown more accurate results were obtained when powdered
samples were attached to a glass slide then compacted as mentioned previously (Dove et
al., 1996).

Surface contamination can also be an issue with the contact angle approach. The glass
slide used in the Wilhelmy plate technique must be free from any impurities and often
contaminants can interfere with the measurement. Contamination of samples can occur
during handling that may influence results. Operator handling can also be another source
of error. Wide ranging results may be obtained from one experiment to another due to
experimental differences and handling issues.

1.15 GRANULATION

1.15.1 Granulation theory

Granulation can be defined as a size enlargement process of a particulate system,
whereby smaller particles are agglomerated into larger, physically stronger units. This
widely used process in the pharmaceutical industry confers many advantages, such as improving content uniformity, enhancing flow properties, densification of the material and improvement in dissolution rate uniformity. Unfortunately, the disadvantages of granulation include an increase in the cost of production and further complexity in understanding each part of the multi-step process.

In spite of its widespread use granulation was often regarded more of an art than a science yet in the last decade great advancements have been made. Progress in understanding the granulation mechanisms has resulted in understanding what outcome a change in a particular variable will confer (Knight, 2004). The aim is to establish an expert system whereby a quantitative change can be predicted within the system as variables as modified. This would enable the knowledge gained from preliminary characterisation of the compound along with granulation theory to accurately define granule properties.

Granulation can be undertaken using a variety of processes, with wet granulation being the most widely used involving the use of granulating fluid to aid agglomeration. Dry granulation involves the compression of particles using mechanical force, either by slugging or roller compaction. Other methods less commonly used include humidification, melt pellitisation and prilling.

1.15.2 Wet granulation

Wet granulation is typically preferred over dry granulation for a number of reasons including providing better control of drug content uniformity at low drug concentrations, control of product bulk density and compactability.

Within the pharmaceutical industry wet granulation is widely undertaken using two types of equipment, fluid bed granulators and high-shear mixers. Fluid bed granulation utilises an upward flow of gas to maintain a continuous movement of particles throughout the batch. Binding solution is often sprayed in an opposite direction to the air
stream and the adhesion of particles to the liquid binder initiates agglomeration. The process of agglomeration and wetting of the particles is a critical step that must be controlled to produce granules with suitable characteristics.

High-shear granulation involves mechanical agitation using impeller blades to rotate the particles within a vessel as binding solution is introduced into the mix. As liquid binder comes into contact with solid particles the first nuclei form to initiate granulation. The mechanical mixing of the wet mass prevents to an extent large agglomerates forming and existing agglomerates undergo densification. The process continues until a stage is reached where further mixing may cause excessive densification or phase inversion. The drying phase is then initiated within a separate vessel or more conveniently within the same vessel. Granules formed by high-shear granulation are generally stronger and denser than those in fluidised bed granulation. Fluidised bed granulation requires fewer handling steps and reduces the time and space needed for granulation. Other processes are used less frequently.

1.15.3 Granulation Process

Traditionally granulation has been described as various combining interactions between the constituent particles and liquid binder. This has led to a number of mechanisms that were variations of one aspect; for example traditionally coalescence and abrasion transfer can be considered as consolidation, Figure 3. It has been more convenient to describe granulation as composing or three distinct sets of rate processes (Tardos et al., 1997). These can be described as wetting and nucleation, consolidation and growth and attrition and breakage as in the modern approach.
1.15.4 Wetting

This is the initial stage of granulation whereby liquid binder will come into contact with dry powder. The addition of binder is an essential step in the granulation process where wetting occurs and initial nuclei are formed. The wetting zone is defined as the area where liquid binder and powder surfaces first come into contact to form initial nuclei and will depend upon the characteristics of the particles within the system. Two aspects are important within the wetting zone, nuclei formation that is a function of thermodynamics and wetting with binder dispersion. Effective mixing of the binder and powder is critical in producing granules that can be controlled and reproducible.
1. INTRODUCTION

1.15.5 Nucleation

Nucleation is the first step in granulation where binder begins to wet the powder and form initial agglomerates. The wetting of the powder from the liquid binder will be driven by the thermodynamics of the system and whether the process is energetically favourable. Wetting thermodynamics have focused on two aspects, the contact angle between solid/binder and the spreading coefficient of the liquid/solid phase. Agglomerates are initially formed when nucleation occurs as the binder begins to wet the powder. Contact angle studies between the liquid binder and solid have been shown to affect the granulated product. As the contact angle of a powder mixture increases using differing proportions of hydrophobic and hydrophilic drug the mean granule size decreases (Aulton and Banks, 1979). Other work has used the spreading coefficient related to the granule properties. The spreading coefficient $\lambda$ is a measure of the tendency of a liquid and solid combination to spread over each other and is related to the works of adhesion and cohesion. Spreading coefficients indicate whether spreading is thermodynamically favourable.

Equation 11 Work of cohesion for a solid: $W_{CS} = 2\gamma_{SV}$
Equation 12 Work of cohesion for a liquid: $W_{CL} = 2\gamma_{LV}$
Equation 13 Work of adhesion for an interface: $W_A = \gamma_{SV} + \gamma_{LV} - \gamma_{SL}$
Equation 14 $W_A = \gamma_{LV} (\cos \theta + 1)$

Where $\gamma$ is the surface energy, $\theta$ is the solid-liquid contact angle and the subscripts L, S, V denote liquid, solid and vapour phase respectively. The work of cohesion is the work required to separate a unit cross-sectional area of a material from itself. The work of adhesion is the work required to separate a unit area from an interface. There are three possibilities that may arise within spreading behaviour: the liquid may spread over the solid ($\lambda_{LS}$), the solid may adhere to the liquid ($\lambda_{SL}$) with no film formation or the solid and liquid will have minimal interaction with the solid-liquid interfacial area at a nominal value. If the spreading coefficient is positive spreading will occur.
spontaneously. Differences in granule properties and prediction of liquid binder have been related to the spreading coefficient (Zajic and Buckton, 1990).

**1.15.6 Consolidation and growth**

Granule growth occurs when individual particles come together and are bound. Whether or not granules collide and form a distinct granule with permanent bonds depends upon liquid binder properties and material properties. Granules can exist in a number of different states and this largely depends upon the liquid saturation of the granule. The mechanisms of bonding in the wet state depend upon capillary and interfacial forces between particles. When liquid binder is added the adsorbed liquid reduces the distance for particle-particle bonding and reduces surface imperfections. Once sufficient liquid is added particles shift from an immobile surface liquid to a mobile liquid film state (Newitt and Conway –Jones, 1958). During granulation it is possible for the saturation state of the granules to shift from the pendular state through to the droplet state, possibly due to addition of liquid binder and/or due to consolidation. The various saturation stages can be described as in Figure 4.

![Figure 1.7 - Schematic of five states of saturation for granular material (From Iveson et.al 2001)](image)

Granule consolidation will be affected by a variety of factors including binder content, binder viscosity, particle size and equipment variables. Greater consolidation will lead to increased densification of particles until a limit is reached. As densification continues granule size and porosity decrease as entrapped air and binder is squeezed to the surface. Porosity is an important property as granules with a high porosity are generally weak and friable. A balance has to be formed between granules that can dissociate with
favourable dissolution characteristics and granules that have sufficient strength to withstand breakage.

1.15.7 Granule breaking and attrition

The breakage of granules can be considered to occur while saturated in the wet state or the fracture of dry granules after processing. Wet granule breakage will often determine the final granule size and attrition of dry granules must be avoided to prevent the production of fine material that may dissociate. An increase in the agitation intensity can reduce the final granule size yet difficulty exists in establishing whether this is caused by a consolidation process or granule breakage (Knight et al., 2000). Wet granule breakage is an important part in granule formation especially in high intensity processes such as high shear granulation and must not be overlooked. Conditions that may initiate granule breakage must therefore not be overlooked.

For example a granule that has high plasticity will deform as a yield stress is exceeded however a brittle material may break under the stress. A relationship between the breakage or non-breakage of granules in a high shear mixer has been described relating the kinetic energy of impact to energy absorbed by plastic deformation (Kenningley et al., 1997). Once the mechanisms of granule breakage are defined the control of final granule size distribution can be achieved.

The attrition of granules following processing, storage, handling or drying is generally undesirable. Breakage of the dry granules will occur as tensile stress is concentrated around cracks within the material and as crack propagation occurs. To measure the fracture properties of a material a number of tests may be applied such as the three-point bend test to determine granule fracture and indentation tests to measure fracture toughness or hardness. The tests can give a better insight into granule breakage so that a prediction of granule breakage for a set material can be made.
In Introduction

There have been great advancements in the understanding of the granulation process. An increase in the understanding of the granulation process has led to knowledge of the resultant effect a change in one or more variables will result in. However, the quantification and control of this change has been the difficulty. This should be the direction for future work whereby the numerous parameters can be quantified.

1.16 Hydrophobic Compounds

Many novel drug compounds have very poor aqueous solubility and pose numerous problems with formulations. A variety of techniques have been used to improve the bioavailability of poorly soluble substances. Milling is a technique often employed to reduce the particle size of a compound to improve dissolution characteristics however, as described earlier can often lead to changes on the surface.

The compounds that were to be used in the study primarily focused on compounds with a very poor aqueous solubility. Another important factor was the ability for the crystalline compounds to exist in the amorphous state. A number of compounds were initially screened for suitability and initially griseofulvin and budesonide were selected. Previous work (Ahmed et al., 1998) has shown that griseofulvin can exist in the amorphous state and be shown to recrystallise with certain water vapour humidity.

1.16.1 Griseofulvin

![Griseofulvin Structure](figure.png)
Griseofulvin is an antifungal drug that has very poor aqueous solubility of $8.624 \times 10^{-3}$ g/l (Elamin et al. 1994). Griseofulvin has a melting point of approximately 220°C and has no known polymorphs or hydrates. It is practically insoluble in water, slightly soluble in ethanol and methanol and freely soluble in dimethylformamide and in tetrachloroethane.

1.16.2 Budesonide

Budesonide is a steroid that also has very poor aqueous solubility of $2.153 \times 10^{-2}$ (Frilink et al. 1991). It has a melting temperature of 230°C, has no known polymorphs and exists in the crystalline state. Budesonide is practically insoluble in water, slightly soluble in acetone and alcohol and readily soluble in dichloromethane.
1.16.3 Indometacin

Indometacin belongs to a class of actives known as the non-steroidal anti-inflammatory drugs (NSAID’s). The compound is practically insoluble in water. The compound is stable in solutions of pH of 7.4 however has rapid decomposition in alkaline conditions.
2. AIMS OF THE THESIS

CHAPTER 2

AIMS OF THE THESIS

The purpose of this project is to:

- Characterise the change in the amorphous form for micronised actives during processing to develop a method to quantify the low levels of disorder initiated by milling.

- Study the changes in the level of disorder as various milling techniques are analysed.

- Investigate the changes of the surface energetic properties of hydrophobic actives that occur during processing.

- Determine the change in the surface properties during storage of compounds and how this may impact the final formulation.

- Study the effect of processing on the granulation characteristics of hydrophobic compounds and the changes that occur to the granulation system due to the presence of disorder in the system.
3.0 METHODS AND MATERIALS

3.1 ISOTHERMAL MICROCALORIMETRY

3.1.1 Introduction

Isothermal microcalorimetry is a technique that measures the change in thermal events to a high degree of sensitivity and can be used to study a range of chemical and biological reactions. Many chemical processes such as a change of phase, a change in the structure or metabolic changes result in a temperature change and may either be exothermic (heat producing) reactions or endothermic (heat-absorbing). The ability to determine the extent of the thermodynamic change can be used to study various reactions particularly in solid state analysis. Isothermal microcalorimetry is based on the heat leakage principle, whereby the heat produced in a thermally defined vessel held under isothermal surroundings results in a temperature gradient. To maintain a balance in temperature heat either flows away from the vessel, in the case of an exothermic reaction or towards the vessel in the case of endothermic reactions. The isothermal microcalorimeter has the ability to detect changes in the sample temperature of one millionth of a degree Celsius. The technique is non-invasive, can be used to analyse chemical or biological systems and is suitable for use for gases, liquids and solids. In solid state applications the technique can be used to study physical form determination such as changes in crystallinity or for stability determination. Applications for the technique used in pharmaceutical analysis have been reviewed extensively (Buckton et al., 1999; Buckton and Darcy 1999).

3.1.2 Instrumentation

The instrument used in the studies was a Thermal Activity Monitor (TAM) 2277, Thermometric AB, Jarfalla, Sweden (Figure 3.1). The instrument comprises of four calorimetric channels, each of which is composed of a twin detector; a sample side (Side A) and a reference side (Side B). The channels are housed within a 25L water
bath and acts as an infinite heat sink. The instrument is capable of operating between 5-80 °C and an external control maintains the temperature at $\pm 2 \times 10^{-4}$. With the accurate control of the temperature and the comparison of the reference and sample side heat differences of $10^{-6}$ are detectable as an out of balance signal.

Figure 3.1 - Diagram of Thermal Activity Monitor (TAM) (reproduced with permission of Thermometric AB, Sweden)
The heat energy from an active in the sample vessel is channelled through very sensitive thermopile conductors which are called the Peltier elements before they escape to the heat sink (Figure 3.2). In this way the sample temperature is maintained at a constant temperature and the surrounding water bath acts as an infinite heat sink. The Peltier elements act as thermoelectric transducers as they convert the differential heat energy into an electrical output using the ‘Seebeck’ effect. The Peltier elements are capable of responding to temperature changes of less than one millionth of a degree Celsius. They are made up from a number of semiconductors that are joined in a series and assembled in a sandwich form. The highly sensitive detectors convert the heat energy into a voltage signal that is proportional to the heat flow. The results are represented as thermal energy produced by the sample per unit of time.

![Diagram of Peltier Elements](image)

*Figure 3.2 - Diagram of Peltier Elements (reproduced with permission of Thermometric AB. Sweden)*

Electrical calibration is conducted by passing through known power values through built-in resistors. Results are shown in the form of power (W) against time which is plotted to show the enthalpic data for the experiments.

An amplifier is connected to each channel, enabling a selection of full-scale measurement ranges between 3 and 3000 µW. The output signal from a thermal event is sent directly through the amplifier to a computer, where the data is collected via the dedicated Digitam software. All data analyses such as the integration of peaks and baseline analysis were carried out using the Microcal Origin software version 7.0.
A range of different reaction vessels are available to fit the calorimetric channel, e.g. closed reaction vessels such as stainless steel or glass ampoules, open reaction vessels including perfusion, titration and flow cells. With these components a wide range of experiments can be carried out.

3.1.3 Experimental

Two different experimental set ups were employed, the first being a closed system (Batch Isothermal Microcalorimetry) and the second an open system (Isothermal RH Perfusion Microcalorimetry).

3.1.3.1 Batch Isothermal Microcalorimetry

All experiments carried out in these studies employed sealed 3ml glass ampoules for both the sample and reference sides of the channel. In each case the reference ampoule was set to match the sample ampoule as closely as possible. In all cases, samples were exposed to an elevated humidity by incorporating a mini-hygrostat filled with the saturated salt solution in a small glass ampoule. The reference side used an empty glass ampoule with the same amount of saturated solution in a mini-hygrostat that was crimped and sealed as that for the sample side.

The ampoules were lowered to the intermediate temperature equilibration position of the channel and the data collection would be initiated. The ampoules were held in this position for 30 minutes until temperature equilibration was complete. The ampoules were then lowered to the measuring site and data recording would continue until the experiment was complete whereby data collection would be stopped and the ampoules removed carefully from the channel. All experiments were undertaken at 25°C.
3. METHODS AND MATERIALS

3.1.3.2 Calibration

Each calorimetric channel was calibrated at least every two weeks, when the amplifier setting was changed or if the experimental conditions were altered i.e., if a different saturated salt solution was used in experiments. Two sealed glass ampoules were employed for this purpose and inserted into each was a mini-hygrostat containing the saturated salt solution to be used in the experiments. A range of amplifier settings (3, 10, 30, 100, 300, 1000 and 3000 μW) were available. An appropriate range was selected according to the data, if no data existed the 3000μW setting was used for initial experiments and lowered to the appropriate setting in further studies. Ampoules were temperature equilibrated for 30 minutes prior to lowering into the measuring chamber. As the baseline signal is constant, the zeroing dial is adjusted so the signal is 0.000 ±0.100 μW. Once this is achieved the second stage can continue, this involves applying an accurate amount of heat by passing a specified current through the channel heater resistor, located in the measuring cup of the channel. The known quantity of thermal energy is recorded as an exothermic response and the deflection from the baseline it adjusted to the correct value (±0.100 μW) by adjusting the fine control dial. The heater is then switched off and the signal is allowed to return to baseline. Once a steady baseline is achieved the zero signal is checked and adjusted using the zeroing dial to 0.000 ±0.100 μW.

3.1.3.3 Isothermal RH Perfusion Microcalorimetry

3.1.3.4 Instrumentation

All experiments using the perfusion microcalorimter used a 4ml stainless steel ampoule that was sealed and connected to the main unit to achieve a closed system. The system as shown in Figure X allows the flow of gas to be controlled by mixing dry gas (0% RH) with wet gas (100% RH) which has been through two humidifying chambers within the unit. The ratio of dry:saturated gas was achieved with a 2281 Precision Flow Switching Valve (Thermometric AB, Jarfalla, Sweden) connected to the computer and the Digitam software using the Flow Switch Module installed on the 2280 TAM Accessory Interface (Thermometric, Sweden). In addition to its RH
controlling function the Flow Switch also incorporated a heater which was employed to prevent condensation forming on the outlet tube. The temperature of the heater was set at 40° C to reduce the occurrence of water condensation interfering with experiments.

Lowering of the RH perfusion unit to the measuring site of the calorimetric channel was carried out in three stages, using a circular clamp to hold the unit as illustrated in Figure 3.3. An empty stainless steel 4ml reference ampoule was used through the studies. The humidifying chambers would be flushed through with the appropriate solvent each week (0.5ml in both chambers).

Figure 3.3 – Schematic diagram of Isothermal RH perfusion unit (reproduced with permission of Thermometric AB. Sweden)
3. METHODS AND MATERIALS

3.1.3.5 RH Perfusion Microcalorimetry Calibration

Calibrations were undertaken a minimum of once a week or if there was any change in the amplifier settings. The amplifier range for the experiment was selected based upon the energy response of the experiment and was selected at 3, 10, 30, 100, 300, 1000 or 3000. The choice was dependent on ensuring the whole response was measured whilst attempting to gain the greatest accuracy by choosing the lowest setting possible, during experiments 300, 1000 and 3000 $\mu W$ were used.

The empty 4ml sample vial was loaded onto the unit and placed in the sample chamber of the TAM. An empty 4ml stainless steel ampoule was used as the reference in all experiments. Both ampoules were held in the temperature equilibration stage prior to lowering in the measuring site. Calibration was conducted with the flow rate set to experimental conditions of 2ml per minute and 0% RH. Once a steady baseline had been achieved the baseline was corrected to read as close to 0 $\mu W$ as possible ($\pm 0.1 \mu W$) by using the zeroing dial on the TAM. Once the baseline achieved $\pm 0.1 \mu W$ at a reading of 0, electrical calibration was undertaken at the specified amplifier range. The heater would then deliver the set amount of power by switching on electrical calibration in the Digitam software. Observations were made and once the signal reached a plateau the ‘fine’ control settings were adjusted to the value required for that particular amplifier range. Once the plateau reached within $\pm 0.1 \mu W$ of the value the electrical signal was switched off. Once the signal had returned to zero and a steady baseline achieved, the value was checked and adjusted if necessary to 0 $\mu W \pm 0.1 \mu W$ by using the zeroing dial as previously. Calibration would then be complete and sample may be loaded into the vial for analysis.

On a monthly basis regular calibration of the RH generated in the perfusion unit was tested. This ensured that the RH being read by the detectors was accurate and that an accurate RH was maintained during experiments. Two saturated salt solutions were placed in the reference and sample vials. The nitrogen flow was switched off and the perfusion unit allowed to equilibrate in the measuring chamber. This would prevent the flow of the dry line of nitrogen though the chambers and into the ampoule. Once
a baseline was achieved and zeroed, the value of the expected RH (of salt solution) was entered into the software and the nitrogen flow switched on. The signal was then stabilised and once a steady level had been reached the adjustment could be made. If the signal was below 0 μW power output the RH was manually increased until the plot crossed the y-axis at zero in 1% increments. The opposite was undertaken if the signal was above 0 μW. The RH was increased and decreased in a similar manner three times to determine the average RH at which it crossed the y-axis. This would be the actual RH value required (on the perfusion setting) to provide the relative humidity that the salt solution produced. This was conducted for both salt solutions and if there was a difference of ±10% of the true value the manufacturers were contacted.

3.2 X-RAY POWDER DIFFRACTION

3.2.1 Introduction

X-rays are electromagnetic wavelengths that occur in the region between gamma rays and the ultraviolet region and have a wavelength between the range of 10 and 0.01 nanometers. Solid state analysis of crystalline material can be undertaken using x-ray powder diffraction (XRPD). Each crystalline solid has its unique characteristic x-ray pattern that is used to identify a species. The basis of the method uses the Bragg’s equation,

\[ n\lambda = 2d \sin \theta \]

Where \( n \) is an integer, \( \lambda \) is the wavelength of x-rays, \( d \) is the spacing between the planes in the atomic lattice and \( \theta \) is the angle between the incident beam and the scattering planes as also highlighted in Figure 3.4.
3. METHODS AND MATERIALS

3.2.2 Instrumentation

The x-ray diffraction system, Phillips PW3710 is composed of a number of functional parts that are co-ordinated by a central computer. A diagram of the system is shown in Figure 3.5.

Figure 3.4 – Schematic diagram highlighting the Bragg diffraction through a cubic crystal

Figure 3.5 – Flow diagram of x-ray diffraction system and the interaction of components in the system
X-rays are produced by generating electrons and accelerating them towards the x-ray tube anode using a high voltage. This can generate large amounts of heat and the water cooler is used to maintain the temperature of the equipment. The spectrum shape and intensity of the beam needed is determined by the voltage and current from the HT-generator as well as the anode material and type of focus of the x-ray tube used. The HT-generator provides the high voltage for the x-ray tube and contains the system used to control the shutters and safety switches.

The x-ray tube is housed in a tube shield that is equipped with shutters for safe operation. The shutters are controlled by the safety circuits in the HT-generator and the diffractometer control unit. These safety circuits ensure that the shutters cannot be opened unless the system enclosure or the radiation shield is closed correctly.

In the case of the experiments a Cu anode has been used. This is suitable for standard applications. The focus type is long fine focus (LFF) used for optimum resolution combined with high intensity; good for phase analysis, non-ambient work and thin film analysis and also for combined point and line focus applications where the resolution provided by point focus is less important. The max kV is 60, max mA is 55 and the max power is 2200W. The advised kV is 40 and the advised mA is 55 that were used in all experiments.

The primary β-filters are located in the primary β-filter disk mounted on the tube shield exit. They are used to monochromate the primary beam, since a monochromatic beam is essential for XRPD experiments. The unwanted x-rays (white radiation plus K-β radiation) are absorbed more than the required (K-α radiation) x-rays. The β-filter in the PW3710 XRD is Ni.

A divergence slit is used in a line focus experiment. The divergence slit in combination with the physical position of the goniometer θ-axis determines the length of the sample that is illuminated. The length of the sample that is illuminated decreases with decreasing slit size and with an increasing value of θ.
The x-rays are focused onto the sample where if a suitable crystalline arrangement comes into contact they are diffracted through a single line through the receiving slit at Brag angle $\theta$. The x-rays pass through the second parallel slit system a scatter slit and a monochromator. The beam then reaches the detector where the signal is recorded as an output of intensity as a function of scan angle $2\theta$ as a diffractogram. The diffractogram can be used for qualitative purposes to elucidate the crystallinity of a compound or used quantitatively by extracting peak information.

![Figure 3.6a](image1)
![Figure 3.6b](image2)
![Figure 3.6c](image3)

**Figure 3.6 – X-ray diffractograms of crystalline, partially amorphous and amorphous material**

Crystalline samples are indicated by well defined peaks and a flat baseline between the region of $3^\circ$ and $40^\circ$ $2\theta$ (Figure 3.6a). Partially amorphous samples have the appearance of peaks not well defined with a broad baseline that is not distinctively flat. The diffractogram can also have superimposed peaks (Figure 3.6b). X-rays pass through amorphous material and this is noticed on the diffractogram as a ‘halo’ pattern with no recognisable peaks and a broad baseline (Figure 3.6c).

### 3.2.3 Sample loading

The instrument should be on standby for at least two hours prior to any experiments being undertaken. The kV was increased one switch position at a time with 30 second intervals between each step to the specified value (40kV). The mA was increased one switch position at a time with 30 seconds between each step to the
3. METHODS AND MATERIALS

specified value (55mA). The instrument was left for 30 minutes before any experiments were conducted. Runs were undertaken from a start angle of 5° 20 to 75° 20 with a step size of 0.02 20 and step time of 2 seconds per step. The sample was placed in the sample pan holder, and placed within the instrument. Powders for testing had a particle size <50μm. Large particle size may give rise to preferred orientation and poor adhesion in the sample holder. Samples may be reduced by gentle grinding in an agate mortar. However, if there is any possibility the sample may undergo any changes, such as polymorphic changes, samples were tested before and after particle size reduction.

3.2.4 Calibration

Calibration of the machine was undertaken every month. A standard manufacturer compact was used to analyse the peaks recorded from the compact to limits stated by guidelines for calibration. Along with the peak analysis linearity checks were also undertaken to maintain the peaks were in the correct position and were within the limits stated by the manufacturer. If there was a deviation of the peaks or the linearity from the manufacturer guidelines further maintenance would be required by a trained engineer.
3.3 INVERSE GAS CHROMATOGRAPHY (IGC)

3.3.1 Introduction

Inverse Gas Chromatography (IGC) is a technique whereby probes (adsorbates) of known characteristics are passed through a column of sample material under investigation within a carrier gas at a fixed flow rate. The interaction of the probes with the surface of the material can be used to calculate properties of the sample such as surface energy, heat or sorption and phase transition temperatures. These calculations are made by correlating the probe molecule characteristics with the retention volume of the probe $V_n$. The technique was developed from gas chromatography and developed so that the material under investigation is packed into a column as opposed to the probe being fixed as in GC. A diagram of the two techniques is shown in Figure 3.7.

![Figure 3.7 - Schematic diagram of the comparison between gas chromatography and IGC (reproduced with permission of Surface Measurement Systems)](image)

3.3.2 Instrumentation

The IGC is composed of many components housed within a single unit of operation. The Inverse Gas Chromatograph Analyser (Surface Measurement Systems, London, U.K) Model SMS-IGC Serial number 010102, Flow control version 1.6 was used for all experiments. The IGC consists of a control PC, the flow control module which regulates the gaseous probes, the probe oven which maintains the probes at a set
temperature and the sample oven which maintains the sample at a specified temperature. A schematic of the IGC is shown below in Figure 3.8.

![SMS/GC Schematic](image)

Figure 3.8 Schematic cutaway of the IGC components (reproduced with permission of Surface Measurement Systems)

The IGC consists of six mass flow controllers that regulate the flow of the gas through the system. The valve system controls the entry and release of vapour gases through the system. Helium is the carrier gas used to transport the probes through the system and is present as a cylinder externally to the system. There are two precise detectors used in the experiments; the thermal conductivity detector (TCD) detects the alkanes and the humidity in the system and the flame ionisation detector (FID) determines the characteristics of the probes after passing through the sample. The FID uses a hydrogen-air flame to burn the probes to elucidate the concentration passing through the column.

### 3.3.3 Methodology

#### 3.3.3.1 Column silination

Prior to active packing it was paramount that columns were silanated to ensure the inner surface of the columns would not interact with the probes used in the experiment. Columns were silanated with Repelcote (dimethyldichlorosilane in
octamethylcyclotetrasiloxane) for 10 minutes, washed with ethanol and rinsed with distilled water. The columns were then held vertically in a beaker and allowed to dry for 24 hours.

### 3.3.3.2 Column packing

Columns were packed on one end of the column with silanated glass wool to create a barrier once the active was introduced. It was paramount the glass wool was packed sufficiently well to prevent the plug from escaping once the carried gas and probes were introduced. The active was then weighed into the column and carefully packed, ensuring there were no loose regions where powder flow could occur. Cracks or poor powder packing can cause errors in the experiments due to powder movement and an alteration of surfaces exposed.

Further detail on the calculation of the surface properties of the material under investigation are highlighted in Section 5.3.

### 3.3.4 Calibration

The system would be regularly maintained and an inorganic reference material namely α-aluminia was used as a system suitability test in the IGC. This would ensure the dispersive surface energies were within the expected range of between 30-35 mJ/m$^2$. Approximately 1g of α-aluminia was packed into a silanised column and conditioned at 25°C and conditioned for 2 hours at 0% RH prior to analysis with a flow rate of 10 sccm. Any large deviation from the expected dispersive surface energy would require maintenance from the manufacturer.

### 3.4 DIFFERENTIAL SCANNING CALORIMETRY (DSC)

Differential scanning calorimetry (DSC) is a technique that measures the change in heat of a material as a function of temperature change. The technique is used to
characterise physical and chemical events by measuring the changes in the enthalpy of a material as a linear or isothermal temperature is applied. Thermal events can be detected as endothermic (i.e., melting, fusion, desorption), exothermic (i.e. crystallisation, adsorption) or heat capacity changes can be observed (i.e. glass transitions). Figure 3.9 illustrates a DSC trace and three types of events are clearly highlighted.

![Diagram of DSC trace highlighting the glass transition, crystallisation and melting events.](image)

**Figure 3.9** – Diagram of DSC trace highlighting the glass transition, crystallisation and melting events.

### 3.4.1 Instrumentation

The experiments were undertaken on power compensated DSC systems with Pyris 1 able to scan at higher scan rates than the conventional scan rates used with DSC 7. Power compensated DSC comprises a system that consists of two isolated furnaces operating on the sample and reference sides (Figure 3.10). The differential temperature between the sample and reference is kept at zero as the power to maintain the same temperature in the two sides is measured. Once an enthalpic change is observed such as crystallisation the temperature changes from the balance with the reference side and power is supplied to the furnace to maintain isothermal
conditions between the two. The DSC delivers a measured quantity of power to the sample and when plotted against temperature a thermoanalytical curve can be constructed using the following equation:

Equation 3.2 \[ P = \frac{dQ}{dT} \]

Where \( P \) (W) is power, \( Q \) is the heat supplied per unit time \( t \).

![Diagram of DSC power compensated design](image)

Figure 3.10 – Diagram of DSC power compensated design

### 3.4.2 Methodology

The experiments were undertaken under two different types of heating rate. Conventional scanning rates were used for samples for crystalline sample and wholly amorphous samples to indicate purity of amorphous samples. HDSC was used for samples that were composed of mixtures and for milled samples where it has been observed the scanning rates can identify thermal events to a greater extent than lower scanning rates. Samples were weighed onto aluminium non-hermitically sealed sample pans (Perkin Elmer) using a microfine balance. A sample mass of 1-3mg were weighed for the actives which provided optimum thermal conductivity. The sample mass was weighed in the centre of the pan and any large aggregates were broken and flattened onto the pan to ensure thermal contact. Parameters such as the heating rate, starting temperature, end temperature and isothermal method were selected according to the active. All experiments were completed in triplicate.
3. METHODS AND MATERIALS

3.4.3 Calibration

Calibration of the instrument was undertaken using IUPAC standards. Indium with a melting temperature of 156.60°C and zinc, 419.47°C were used as the calibration standards. Calibration would be undertaken on a weekly basis and conditions were as those used in the experiments. A change in the heating rate would require re-calibration and this ensured accuracy of experiments. Calibration of the furnace and removal of any contaminants were undertaken on a regular basis. The furnace would be calibrated and cleared of any debris on a regular basis.

3.5 Preparation of amorphous active

The amorphous standard was prepared by quench cooling of the crystalline material. Approximately 5g of crystalline active was heated to the melting temperature in a porcelain crucible and wire heated over a wire gauze using a Bunsen burner. At the onset of melt the liquid active was poured into a flask containing approximately 100ml of liquid nitrogen. The sample was rapidly cooled and the excess liquid nitrogen removed. The active formed spherical particles which were lightly crushed using a mortar and pestle and passed through a 500µm brass Endecotts sieve. Samples were then placed within a dessicator containing P₂O₅ and held at 0% RH for 2 hours before analysis to remove any moisture from the sample. Samples would be prepared for use on the particular day so any changes to the crystallinity of the active were negligible.

3.6 Preparation of physical mixtures of amorphous /crystalline active

Physical mixtures of amorphous/crystalline w/w active were prepared by weighing the crystalline material into a glass ampoule. Amorphous material would be accurately weighed into the vial for the specific ratio required. The mixture would be mixed for 10 minutes in a Turbula mixer prior to use. Samples that were to be investigated were prepared on the day of analysis.
3.7 Preparation of the saturated salt solutions for maintaining specific RH conditions

A salt was added into distilled water maintained at 60°C under continuous stirring to obtain a saturated solution; water temperature (60°C) and constant stirring was maintained using a hot plate with a magnetic stirrer as described by Nyqvist, 1983. The saturated salt solution was then cooled to ambient temperature and poured into a glass dessicator. Each salt lowers the vapour pressure of water to a different extent and hence produced a different RH atmosphere in the sealed dessicator. A table of the RH values of each salt reported (Nyqvist, 1983) is shown in Table 3.1.

<table>
<thead>
<tr>
<th>Salt</th>
<th>Temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>20°C</td>
</tr>
<tr>
<td>Lithium Chloride, LiCl</td>
<td>11.3</td>
</tr>
<tr>
<td>Magnesium Bromide, MgBr₂</td>
<td>30.8</td>
</tr>
<tr>
<td>Sodium Dichromate, Na₂Cr₂O₇</td>
<td>54.8</td>
</tr>
<tr>
<td>Sodium Iodide, NaI</td>
<td>39.5</td>
</tr>
<tr>
<td>Magnesium Nitrate, Mg(NO₃)₂</td>
<td>54.5</td>
</tr>
<tr>
<td>Sodium Bromide, NaBr</td>
<td>59.5</td>
</tr>
<tr>
<td>Copper Chloride, CuCl₂</td>
<td>67.7</td>
</tr>
<tr>
<td>Potassium Iodide, KI</td>
<td>69.9</td>
</tr>
<tr>
<td>Sodium Nitrate, NaNO₃</td>
<td>75.4</td>
</tr>
<tr>
<td>Sodium Chloride, NaCl</td>
<td>78.5</td>
</tr>
<tr>
<td>Potassium Chloride, KCl</td>
<td>85.1</td>
</tr>
<tr>
<td>Potassium Nitrate, KNO₃</td>
<td>94.6</td>
</tr>
<tr>
<td>Potassium Sulphate, K₂SO₄</td>
<td>97.6</td>
</tr>
</tbody>
</table>

Table 3.1 – Table of the relative humidity of saturated salt solutions at the specified temperature.
3.8 MICRONISATION

3.8.1 Air jet mill

The GEM-T fluid energy Trost Mill was used to mill samples using a compressed air supply. The microniser uses the principles of the Venturi effect where the movement of gas at high pressure results in size reduction of particles occurs through particle impact and attrition. Particles are fed through the mill and cycle through a circular motion as the drive pressure (P jet) forces particle into the mill. The grind pressure from the O jet causes bombardment of particles within the impact chamber where particle attrition and impact occur. The energy initiates particle fracture and as particles are reduced they move towards the discharge chamber and are collected in a glass container. Figure 3.11 shows the various components of the air jet mill. The feed rate was approximately 1 gram per minute and the inlet pressure was varied, the O jet pressure would not exceed the P jet pressure as to prevent backflow and were varied though the experiment. Samples were fed through and collected from the depositing chamber. Approximately 10 grams samples were air jet milled and placed within a 0% RH desiccator until analysis was undertaken.

Figure 3.11 – Schematic of Trost Gem-T air jet microniser
3.8.2 Fritsch ball mill

The Fritsch high energy rotary mill was used to initiate particle size reduction. Approximately 10g of active was weighed into a 500ml sintered corundum vessel with 15 agate balls of 15mm diameter as recommended by the manufacturer guidelines. Samples were clamped into the mill and the time and revolutions were set to achieve particle size reduction. Samples were milled at 300rpm over a period of 1, 5, 10 and 24 hours and samples were placed in a P2O5 dessicator for analysis on the day milling would be completed.

3.8.3 Vibration Ball Mill

The vibration ball mill used in the experiment is the 5100 SPEX CertiPrep Mixer/Miller used with an agate vial set 6133 with methacrylate balls, 3/8 inches. The mill operates along three axes where a multi-directional force initiates grinding. The grinding medium covered approximately 50% of the vial. Samples of approximately 1 gram were placed within the vial just covering the grinding medium to ensure maximum grinding. Samples were ground in 1 minute sequential time periods, due to agglomeration on one side of the vial. After 1 minute periods the powder sample was mixed.

3.9 Wet granulation

Granulation was undertaken using a Kenwood Chef Mixer to mix the constituents in batches of 100g. The bulking agent and active were weighed and added to the stainless steel mixing bowl and dry mixed for 15 minutes at speed setting 1 (the lowest speed setting). The binder solution was made as a 5% w/v solution and added to the solution at a rate of 1ml/minute using a Dosimat pump to ensure a measured amount of binder added to the mix. Mixing would continue as binder addition was undertaken until 10ml of binder solution was added. Mixing would be stopped and the wet mass lightly sieved through a 1.2mm sieve to remove any aggregates on a stainless steel tray. Granules were tray dried in a temperature controlled oven at
40°C for 4 hours until all granules were sufficiently dried. Granules were passed through a 1.2mm sieve to break any large agglomerates and stored in 500ml amber bottles until further experiments were undertaken.

3.10 Drying

Granules were tray dried at 40°C for five hours in a temperature controlled oven. The granules were weighed every 30 minutes and once there was less than a 5% change in weight the granules were removed from the oven. Large agglomerates were broken by lightly sieving through a 2mm stainless steel Endecotts sieve. The granular material was then stored under ambient conditions in 500ml glass amber bottles.

3.11 Binder addition

The binder used in all granulation experiments was polyvinylpyrrolidone (PVP) and binder was added using a Dosimat pump drop wise as granulation proceeded. The rate at which binder was added was continuous throughout the experiment and was set at a rate of 1.0 arbitrary units.

3.12 Granule sizing

Granule particle size was measured using an increasing set of brass Endecotts sieves of aperture sizes of 0.045, 0.075, 0.12, 0.18, 0.25, 0.3, 0.5, 0.71 and 1mm. Approximately 50g of granular material was placed at the top of the nest of sieves and placed onto a Copley sieve shaker for 10 minutes at an amplitude of 30. The granules at each level was weighed on an Orion Cahn Autobalance AD4 (Thermo Scientific, Germany) and granules were returned into the 500ml amber bottles.

3.13 UV spectrophotometer
The use of ultra violet light is a direct way to measure the concentration of any substance exhibiting a chromophore. A chromophore is defined as a functional group that can absorb light in the UV region. This property enables the determination of the concentration of a drug in solution and is commonly used during drug dissolution tests to measure the amount of drug released in a given time. The spectrophotometer measures the intensity of UV light after passing through the solution. If the solute in solution absorbs light at a specific wavelength then the intensity before and after passing through the solution should change. This ratio can be used to calculate the concentration as follows by using the Beer-Lambert law,

\[
A = \log \frac{I}{I^0} = \varepsilon cl
\]

Where \(A\) is the measured absorbance, \(I^0\) is the intensity of the incident light at a given wavelength, \(I\) is the transmitted intensity, \(l\) is the path length and \(c\) is the concentration of the absorbing species and \(\varepsilon\) is a constant known as the molar absorptivity coefficient.

A spectrophotometer consists of a light source, a monochromator to transform the light into a light of single wavelength and a filter which is used with the monochromator to filter out other wavelengths. In the experiments a Cary 3E UV-Visible Spectrophotometer (Varian, Palo Alto, CA, U.S.A) was used to measure the concentration of the actives in solution. The solution sample was passed through 0.45 \(\mu\)m filters and 5ml of the filtrate was added into quartz cuvette and analysed.

### 3.14 In vitro dissolution

#### 3.14.1 Calibration Curve

20mg of the active was accurately weighed and dissolved in a volumetric flask and made up to 1L with pH 6.8 phosphate buffer solution. The solution was manually mixed for 10 minutes and gradually diluted from stock solution to give a concentration range from 1mg/1000ml to 20mg/1000 ml. The nine points of concentration was measured using the Cary 3E UV-Visible Spectrophotometer
(Varian, Pal Alto, CA, U.S.A.) at 295 nm for griseofulvin and 320 nm for indometacin. The absorbance was tested in triplicate for each concentration level and averaged to construct a calibration curve that would enable the quantification of the concentration in solution.

### 3.14.2 Dissolution test

The dissolution experiments were carried out using a Pharma Test dissolution apparatus (Pharma Test, Hainburg, Germany) that was connected to a Cecil UV-Vis spectrophotometer (Cecil CE 2020, Cecil Instruments, Cambridge, U.K) to analyse the samples. 900ml of degassed dissolution medium was poured into each one of the six vessels and warmed to 37°C before starting the experiment. The paddles were set 25 ±2mm from the bottom of the vessel to ensure they met BP specifications and rotated at 50 rpm. Samples were automatically taken using the control settings in the dissolution software at 5 minute intervals after passing through 0.45 μm disposable filter units. The absorbances of the samples were measured at 320 nm for indometacin. Results were recorded as an average of three separate experiments.

### 3.15 Friability test

The friability of the granules was analysed by weighing 10g of the granules (250-1000μm) together with 50 methylacrylate balls (mean diameter 1mm) an a Pharma Test-type friabilator (Pharma Test, Hainburg, Germany) for 10 minutes at a rotational speed of 25 rpm. After the rotations have completed the methylacrylate balls were removed and the granules were sieved over a 250 μm screen. The friability % was calculated using the following equation:

\[
\text{Equation 3.4} \quad \text{Friability} \% = 100 \times \frac{(P - P')}{P}
\]

Where P is the initial weight of the granules (10g) and P’ is the final weight after sieving over a 250 μm screen.
### 3.16 MATERIALS

<table>
<thead>
<tr>
<th>Material</th>
<th>Supplier</th>
</tr>
</thead>
<tbody>
<tr>
<td>Budesonide</td>
<td>300087-01 AstraZeneca U.K.</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>Sigma-Aldrige, Poole, U.K.</td>
</tr>
<tr>
<td>Griseofulvin</td>
<td>043K1396 Sigma-Aldrich U.K.</td>
</tr>
<tr>
<td></td>
<td>G4753-25G Molekula U.K.</td>
</tr>
<tr>
<td>Lactose</td>
<td>B23416 Borculo Whey U.K.</td>
</tr>
<tr>
<td>Pharmatose 200M</td>
<td></td>
</tr>
<tr>
<td>Polyvinyl pyrildone</td>
<td>Sigma-Aldrige, Poole, U.K.</td>
</tr>
<tr>
<td>Microcrystalline cellulose</td>
<td>Sigma-Aldrige, Poole, U.K.</td>
</tr>
<tr>
<td>Magnesium Stearate</td>
<td>U00714 Sigma-Aldrich, Poole U.K.</td>
</tr>
<tr>
<td>Phosphorous pentoxide</td>
<td>Sigma-Aldrige, Poole, U.K.</td>
</tr>
<tr>
<td>Ethanol (HPLC grade)</td>
<td>BDH, Leicestershire, U.K.</td>
</tr>
<tr>
<td>Decane (HPLC grade)</td>
<td>Fisher Chemicals, Loughborough, U.K.</td>
</tr>
<tr>
<td>Nonane (HPLC grade)</td>
<td>Fisher Chemicals, Loughborough, U.K.</td>
</tr>
<tr>
<td>Octane (HPLC grade)</td>
<td>Fisher Chemicals, Loughborough, U.K.</td>
</tr>
<tr>
<td>Heptane (HPLC grade)</td>
<td>Fisher Chemicals, Loughborough, U.K.</td>
</tr>
<tr>
<td>Hexane (HPLC grade)</td>
<td>Fisher Chemicals, Loughborough, U.K.</td>
</tr>
<tr>
<td>Acetone (HPLC grade)</td>
<td>Fisher Chemicals, Loughborough, U.K.</td>
</tr>
<tr>
<td>Chloroform (HPLC grade)</td>
<td>Fisher Chemicals, Loughborough, U.K.</td>
</tr>
<tr>
<td>Ethyl Acetate (HPLC grade)</td>
<td>Fisher Chemicals, Loughborough, U.K.</td>
</tr>
<tr>
<td>Zinc (99.99% purity)</td>
<td>Sigma-Aldrige, Poole, U.K.</td>
</tr>
<tr>
<td>Lead (99.99% purity)</td>
<td>Sigma-Aldrige, Poole, U.K.</td>
</tr>
</tbody>
</table>

*Table 3.2 – Materials used during experimental analysis with supplier and batch numbers*
CHAPTER FOUR

THE QUANTIFICATION OF MICRONISED HYDROPHOBIC ACTIVES USING ISOTHERMAL MICROCALORIMTERY
4. RESULTS AND DISCUSSION

4.1 INTRODUCTION

Many new active pharmaceutical ingredients (API's) have a poor aqueous solubility that can often be a limiting factor in early stages of development. This can present a challenge in the development in a formulation that is administered as a solid dosage form. There are many approaches that can be implemented to improve the bioavailability of API's, the most simplest and common being particle size reduction. The Noyes-Whitney equation as discussed previously shows how decreasing the particle size leads to an increase in the particle surface area with a subsequent increase in the rate of dissolution. If the particle size distribution of the API cannot be controlled or altered during the crystallisation process, size reduction is often undertaken via a high-energy physical process such as milling. The particle size reduction during milling is dependent on a number of factors including the milling equipment, initial particle size and properties of the API that influences the stress imparted on the crystalline structure. Unfortunately, the energy required to disrupt the lattice into breakage can generate large areas of disorder on the surface and if extensive can result in large proportions of the API as amorphous regions.

API's that possess large quantities of disorder have shown to have increased dissolution rates (Chiou and Kyle 1979; Hendrikson, 1990), decreased chemical stability (Otsuka and Kaneniwa, 1990) and differing compaction properties (Sebhatu et al., 1994a). Even if only the surface layers are affected it may still have a pronounced effect on the physico-chemical properties since many particle characteristics depend on the structure and state of the surface. As partially amorphous surfaces develop during milling the physical properties will subsequently alter to that of the crystalline material and will have various degrees of disorder present at the surface (Dialer and Kuesner, 1973; Saleki-Gerhardt et al., 1994; Ward and Schultz, 1995). The amorphous state is metastable with respect to its crystalline equivalent and can have a major influence on the performance of a system during processing (Elamin et al., 1994). With current process analytical technology (PAT) guidelines it is very important to be able to understand variations during process manufacture and to understand how these changes can influence behaviour of API's through the various stages of development. Analytical techniques must be able to
4. RESULTS AND DISCUSSION

accurately quantify the indiscrete regions that may only represent a small percentage in terms of mass.

This poses a problem using traditional analytical techniques such as X-Ray Powder Diffraction (XRPD) and Differential Scanning Calorimetry where the limit of detecting amorphous material is often 5-10% (Giron et al., 1997). Isothermal microcalorimetry is a technique that can be used to show changes in the surface of an active and has been used to quantify low levels of disorder from as low as 1% (Briggner, 1994; Sebhatu 1994b and Buckton, 1995). A detailed review of the techniques for the assessment of the disorder showed the mini-hygrostat method and the advantages and disadvantages with other methods (Buckton and Darcy 1999). This technique has advantages of quick analysis, real time power time data and the sealed environment can be used to study chemical processes occurring within the chamber. There are numerous disadvantages with this technique; firstly there is a minimum thermal equilibration time once the experiment has been initiated. There is also the inability of the apparatus to control the release of solvent and the apparatus is unable to control the flow through the chamber. The perfusion unit is an apparatus that can control the flow of solvent and control the relative humidity (RH) in an experiment. This has been used to quantify low levels of amorphous content in hydrophobic actives and can measure the amorphous content to as little as 1% (Ahmed et al., 1996) and more recently from 0.5% (Mackin et al., 2001) using perfusion microcalorimetry.
4. RESULTS AND DISCUSSION

4.2 AIMS

The initial aim was to develop a technique to quantify low levels of disorder for hydrophobic actives using organic vapour to induce crystallisation. Thee hydrophobic actives; griseofulvin, budesonide and indometacin were analysed and calibration curves constructed so amorphous content could be studied accurately.

A further aim was analyse the best method for the quantification of amorphous content. The re-crystallisation dynamics are complicated in terms of the many processes occurring within the perfusion unit with many differences to that of an established method such as batch microcalorimetry.

Finally the hydrophobic actives were milled using three differing techniques. A rotary ball mill, air jet mill and high vibration ball mill were used to increase disorder within the actives to test differences in amorphous content with a variation in milling technique. This disorder would then be measured using the most accurate quantification technique as described for the second aim in order to determine the extent of amorphous content induced by the techniques. Variability such as milling pressure and time will also be investigated to determine what effect a change in the variables can have on the amorphous content.
4. RESULTS AND DISCUSSION

4.3 EXPERIMENTAL METHOD

4.3.1 Preparation of crystalline samples

Crystalline actives were milled and exposed to a 100% p/p$_0$ ethanol vapour for 12 hours. The crystalline material was sieved through a 100 µm Endecotts sieve. This was to ensure crystalline fines resembled particle characteristics more closely to that of the milled material that would be studied. Samples were dried over Phosphorous Pentoxide (P$_2$O$_5$) for 12 hours to ensure the material was sufficiently dried prior to use in experiments.

4.3.2 Preparation of amorphous samples

Amorphous material was prepared by quench cooling as outlined in Section 3.5. Amorphous material was sieved through a 100 µm Endecotts sieve and stored in P$_2$O$_5$ under vacuum (-300 mbar) prior to use. Amorphous samples were prepared prior to analysis and stored for a minimum time to limit any possible crystallisation.

4.3.3 Preparation of micronised samples

Micronised samples were prepared using one of three milling techniques as outlined in Section 3.8. Once samples were milled they were stored in P$_2$O$_5$ under vacuum (300 mbar) prior to use. Samples would be milled on the day of analysis.

4.3.4 Isothermal Batch Microcalorimetry

The isothermal batch microcalorimetry method (as mentioned in Section 3.1.3.1) was used to determine the amorphous content of physical mixtures of crystalline/amorphous actives w/w. The technique was also tested for use on micronised samples and whether the technique would be suitable to determine the amorphous content of lower amorphous content mixtures. Preliminary experiments
were conducted on the isothermal microcalorimeter using the ampoule method as explained in Section 3.1.3.1.

4.3.5 Isothermal perfusion microcalorimetry

Early work by Bhatt and Rubenstein, 1983 used a gas flow system to measure the interaction of water vapour with various crystalline and amorphous solids. The technique was used to measure very small amounts of disorder on crystalline material by using the vapour from saturated salt solutions to generate the probe gas. This early technique led to the development of the present perfusion microcalorimetry use. The technique has shown considerable effectiveness at highlighting the differences in batch variability from three suppliers of α-lactose monohydrate (Sheridan et al., 1995). The technique enabled variability in the surface energetics where contact angle measurements failed to measure this difference. Further developments have shown the technique capable of measuring the amorphous level of disorder from 0.5% w/w for the amorphous content of a micronised benzyl ether derivative (Mackin et al., 2001). The methods for the perfusion unit are applied however the only criteria are that the amorphous material will recrystallise on exposure to solvent vapour and no hydrates or solvates are formed.

The Isothermal Perfusion Unit was the apparatus used in the experiment to quantify the enthalpy of crystallisation and to study the behaviour of micronised samples. The design and experimental set-up is described in Section 3.1.3.3. Samples between 10-100mg were loaded into the 4ml stainless steel ampoule and placed in the sample side of the TAM. The correct weight of sample was used to keep the experiment within the range of the TAM. The reference chamber was an identical empty 4ml stainless steel ampoule as the sample chamber.

The humidifying chambers were filled with 0.5ml ethanol 96%. The nitrogen gas flow was set at 2ml/min and checked with an external flow meter. The flow controllers directed vapour from the dry line (0% p/p0) or wet line (100% p/p0), the combination of the two would result in the specified % p/p0. The flow switch heater
was set at 40°C to prevent condensation of water. Calibration of the unit was undertaken every five experimental runs. Triplicate control runs of the empty stainless steel ampoule was undertaken to establish the wetting response due to the sample chamber. The average response was subtracted from the actual sample response prior to analysis. The data was then analysed using the Origin software version 7.
4. RESULTS AND DISCUSSION

4.4 GRISEOFULVIN RESULTS AND DISCUSSION

4.4.1 Batch isothermal microcalorimetry results

Initially the crystallisation response of griseofulvin was studied using saturated salt solutions as previously studied by Ahmed et al., 1998. Isothermal batch microcalorimetry experiments as outlined in Section 3.1.3.1 were undertaken to provide a method of rapid analysis of physical mixtures of quenched/crystalline griseofulvin in ratios of 0, 25, 50 and 100% w/w. The mixtures were prepared in glass ampoules and sealed with a mini-hygrostat containing 75% RH saturated salt solution to initiate crystallisation. The ampoule experiments provided a method of rapid analysis with minimal experimental set-up required and enabled the study of the range of the response of crystallisation. The data shown in Figure 4.1 compares the response from the mixtures as the raw data from the TAM of the energy response over time.

Figure 4.1 – Plot of TAM response for physical mixtures of quenched/crystalline griseofulvin 0, 25, 50 and 100% w/w using 75% RH saturated salt using batch isothermal microcalorimetry to initiate crystallisation.
4. RESULTS AND DISCUSSION

On observation of Figure 4.1 the initial 30 minutes of the data are lost as the experiment occurs immediately once the ampoule is sealed and placed into the TAM. The saturated salt solution used in the experiment was NaCl which gave 75% RH within the sealed ampoule at equilibrium. During the temperature equilibration stage, crystallisation has proceeded and results in a large proportion of the data missing. The error is greater for samples with lower amorphous content % w/w in the physical mixtures.

The data was corrected for weight for each individual sample and analysed using the Origin 7.0 software. Baseline correction was undertaken by taking the point at the base of the curve and extrapolating the data to convert the energy response from μW to W and convert to joules per gram for each sample. The wetting response from an average of an empty glass ampoule with the same amount of 75% RH saturated salt solution was subtracted from the values to ensure the ‘wetting’ signal was not included. Averages for triplicate samples were taken and plotted as the enthalpy of crystallisation against the ratios of quenched/crystalline griseofulvin 0, 25, 50 and 100% w/w physical mixtures as shown in Figure 4.2 Taking into account the loss of the initial 30 minutes of data there is still very good correlation ($R^2 = 0.99719$). An underestimation of the 25% mixture can be due to the increase in error as the crystallisation process proceeds during the temperature equilibration stage. There is a slight curve to the relationship that may be attributed to this ‘lost’ data where the loss of data results in a reduced response. The standard error will have a greater impact on lower amorphous mixtures such as that for the 25% amorphous mixture which is highlighted in Figure 4.2.
4. RESULTS AND DISCUSSION

Figure 4.2 - The enthalpy of crystallisation of griseofulvin samples from 0-100% w/w amorphous content using quenched/crystalline mixtures of 0, 25, 50 and 100% amorphous samples crystallised using 75% RH saturated salt solution in batch isothermal microcalorimetry.

The initial experiments provided a benchmark to investigate the response from a range between 0 to 100% amorphous content w/w to determine whether the technique is suitable for measuring lower amorphous content mixtures w/w. Figure 4.2 displayed the error when measuring lower amorphous content at 25% however many milled samples exhibit amorphous content between 5-10% w/w. The batch isothermal microcalorimetry method could be used for experiments where there is a longer lag time prior to crystallisation however was not accurate for lower amorphous content mixtures. The method was used for micronised samples and the comparison between the method and the perfusion method can be shown in Figure 4.3.
4. RESULTS AND DISCUSSION

During the batch isothermal microcalorimetry experiments as indicated by the red line in Figure 4.3 at 2 hours the ampoule is in the measuring site of the isothermal unit. The ampoule must remain in the temperature equilibration stage for at least 30 minutes prior to lowering to discount any thermal events recorded due to differences in temperature. As the unit is lowered after 30 minutes the reaction proceeds in contrast to the dynamics of the perfusion unit. The black line in Figure 4.3 illustrates the reaction and proceeds as soon as the solvent vapour comes into contact with the sample. There is thermal equilibration prior to the start of the experiment however the flow is controlled externally so the reaction can begin at time 0 when initial contact between the vapour and solid surfaces occurs. Within the conventional ampoule experiment the reaction has preceded as the solvent is interacting with the sample after sealing. As can be seen in Figure 4.3 the majority of the reaction is not recorded using ampoule calorimetry which was especially the case with lower amorphous content physical mixtures and micronised samples where disorder was primarily located on the surface.
4. RESULTS AND DISCUSSION

4.4.2 Isothermal perfusion microcalorimeter results

The use of the RH perfusion microcalorimetry to calculate the amorphous content of partially amorphous samples overcame many of the difficulties associated with the mini-hygrostat technique. The dynamic nature of the system and the ability to control the flow of the solvent vapour enabled variation in the RH during the experiment. The control enables a steady baseline to be achieved prior to analysis as the sample can be kept at 0% RH which is crucial for partially amorphous samples. Any moisture present on the surface is removed prior to analysis and allows the experiment to begin at time 0 (t₀). Once a stable baseline is achieved the simultaneous recording of the power output can be recorded as the vapour interacts with the surface of the compound. The advantages of the perfusion microcalorimetry apparatus led to the use to determine the amorphous content of milled samples. In the experiments to establish the amorphous content ethanol (HPLC grade) was used as the solvent vapour to induce crystallisation. Limitations when using water to induce crystallisation in budesonide samples led to the use of using organic solvents to induce crystallisation. Previous studies have used (Ambarkhane et al., 2006, Ahmed et al., 1996) ethanol to investigate the crystallisation of hydrophobic actives.
4. RESULTS AND DISCUSSION

Figure 4.4 shows an example of a physical mixture of griseofulvin consisting of 5% w/w amorphous mass from a 50mg sample. After samples are loaded into the measuring ampoule the unit is temperature equilibrated for 20 minutes prior to the beginning of the experiment. The first stage of the experiment indicates the lowering of the perfusion unit into the measuring chamber. The lowering is characterised with a thermal event due to the friction of the perfusion unit against the sides of the isothermal microcalorimeter. The reading that is displayed shows a sharp exothermal peak. As the temperature of the ampoule is equilibrated with the TAM this response decreases rapidly. The second stage of the experiment displays the start of when the solvent vapour is released using the flow controllers into the measuring site. As the nitrogen carrier gas takes the solvent vapour to the measuring ampoule, the sample and the stainless steel ampoule are adsorbed with solvent. The isothermal microcalorimeter displays thermal data however does not give any indication of chemical reactions taking place. In Figure 3.4 which shows stage two there is initial uptake of the vapour which is rapid. This peak is the adsorption of the solvent onto the surface of griseofulvin and results in the crystallisation which is stage 3. The presence of a dual peak noted as the second exothermic peak is partly embedded after the adsorption/absorption peak suggests crystallisation occurs. This is sometimes masked and not clearly defined as Figure 4.4 as the reaction results in a larger output which is only shown as one peak. As the peak settles and returns to zero the next stage of the experiment can continue. Stage four is the desorption stage, where the RH is switched from 95% (p/p₀ for ethanol) to 0%, the flow of nitrogen removes the solvent from the surface of the active and results in a sharp endothermic peak.

4.4.3 Differences between ampoule experiments and perfusion unit

Differences exist between the ampoule and perfusion microcalorimetry dynamics as the crystallisation reaction proceeds. In the ampoule experiment a mini-hygrostat has the solvent or salt solution with the sample surrounding the hygrostat that is sealed in
a glass ampoule. The evaporation of the solvent from the hygrostat generates a heat flow which is different from the perfusion unit; where the vapour is generated outside the sample chamber. The perfusion technique also provides a dynamic flow of vapour to the sample, this rapid generation of a flow of vapour results in a much faster wetting and crystallisation response. There must be consideration taken with thermodynamically unstable materials where it can be difficult to determine when equilibrium has been achieved following water sorption. This is due to the fact there is a lag time before a solid state change is observed and is often a problem with experiments that involve a constant rate increase or decrease.

![Graph](image)

Figure 4.5 – Isothermal RH Perfusion microcalorimetry plot of the crystallisation of a quenched/crystalline 5% w/w physical mixture of griseofulvin (----) and air jet milled (80/80 psi) griseofulvin (-----) using ethanol (95% p/p<sub>b</sub>) to initiate crystallisation in a step method of 0-95-0 % p/p<sub>b</sub>.

4.4.4 Amorphous standard

The amorphous standard used in the calibration curve was quenched griseofulvin prepared by supercooling of the melt as outlined in Section 3.5. Griseofulvin was scanned for any impurities using DSC analysis for each batch. Each perfusion experiment lasted 12 hours and each batch of quenched griseofulvin was prepared
prior to every experiment to ensure accurate data, so any change that may occur upon storage were negligible. Milling was the preferred method to create the amorphous standard however, the samples that were milled using the rotary Fritsch ball mill only resulted in a partially amorphous active as indicated by the XRPD trace. Milling was the preferred method since the particle size of the active would be similar to the active that would later be studied during the milling studies. XRPD also showed the most intensive milling did not lead to a wholly amorphous material that could be used as a standard as diffractograms showed the material to be partially crystalline. Spray drying was also attempted however yield was very poor and triboelectrification was disadvantageous for sample handling. To enable an effective calibration curve to be constructed it is important to select the amorphous standard by which enthalpic data can be compared against. Since it was difficult to ascertain the level of amorphicity in the milled samples it was not selected to be used in the calibration curve experiments for each active. Quenched active was used as it provided a standard more representative of a wholly amorphous compound. There were issues with using a standard that did not represent the method by which it is intended to study however this is unavoidable. Particle size issues may also be of concern however quenched samples were gently milled and passed through a 100μm sieve to prevent any large particles causing any differences in the sorption of the solvent.

4.4.5 Physical mixtures

The disadvantage of using physical mixtures was that the system did not truly reflect the system of a micronised sample. Micronised samples have amorphous regions scattered over the surface of the active as an outer layer with the core consisting of crystalline material. Physical mixtures have crystalline components and amorphous components which are not fused as particles and exist as separate particles mixed homogenously. The two systems behave differently however greater accuracy is obtained using this method then using a standard that is partially amorphous and taken as the 100% amorphous standard. From Figure 4.5 there is a comparison between the physical mixtures and the micronised samples. The sharper peak of the micronised sample indicates a greater surface area interaction which is expected due
to the more homogenous particle size. The rate of sorption of the solvent also indicates the amorphous regions on the surface of the material interact rapidly. Crystallisation occurs during sorption of the solvent in a very rapid process. The physical mixtures have a typically bi-modal curve more prominent at higher percentage mixtures with a lower rate of interaction initially. This is due to initial sorption onto the surfaces of the crystalline and amorphous component with the second peak attributed to the crystallisation of the amorphous active. This is the time taken for the solvent to lower the $T_g$ as the amorphous particles take some time before crystallising as the solvent must penetrate the core. This indicates the amorphous particles in the physical mixtures are larger than the amorphous particles in the micronised sample which covers a greater proportion of the surface. Although the kinetic response is different between the two systems the total enthalpy will not be affected.

### 4.4.6 Method to extract the data attributed to the enthalpy of crystallisation

The data from the perfusion microcalorimeter incorporates many processes occurring simultaneously. Once the solvent vapour enters the sample chamber there is initial wetting of the sample and the sample container. If there is any amorphous material crystallisation may occur if the $T_g$ is lowered sufficiently. Physical mixtures that had a very low amorphous content and micronised samples re-crystallised during the initial wetting phase. The data was obscured by the wetting signal and to extract the relevant enthalpy of crystallisation two methods were used to obtain the significant data.

### 4.4.7 Method A – Data extrapolation

Method A used an average triplicate crystalline response for the same mass of crystalline material used in experiments. The average crystalline enthalpic data was subtracted from the sorption curve in the experimental data to eliminate the wetting of the crystalline component and sample container in all experiments. This would leave only the enthalpic response for crystallisation of the active. The enthalpic data
would then be corrected for weight and the physical mixtures would enable the construction of a calibration curve relating the enthalpy (J/g) of the reaction to amorphous content.

4.4.8 Method B – Data extrapolation

Method B involved another stage in the isothermal perfusion unit. After the initial wetting phase, initiated when the solvent vapour is maintained at 95%RH, the RH would then switch to 0% RH so that a drying phase is established. This method is sample specific and takes into account the wetting of the same sample under investigation. There is a difference between comparing the wetting of the crystalline material after it has crystallised to the material before wetting has occurred, yet this is unavoidable. The two methods provide the best means for extracting the relevant data. Calibration curves were constructed using the two methods for each active under investigation.

4.4.9 Griseofulvin perfusion isothermal microcalorimetry data

4.4.9.1 Griseofulvin calibration curve Method A results
4. RESULTS AND DISCUSSION

Figure 4.6 – Calibration curve using data analysis of Method A for physical mixtures of quenched/crystalline griseofulvin from 0.5% to 5% w/w amorphous content.

The results of the calibration technique applied to the results using Method A are displayed in Figure 4.6. A number of points can be expressed about the nature of the relationship between the final enthalpic data that is extrapolated. Firstly taking the crystalline sample with an assumed 0% amorphous quantity it can be shown that the value is above 0 J/g, specifically 1.25 J/g of enthalpy indicating a difference between the measured values and the average crystalline response. The mean enthalpy of three crystalline samples of 50mg were measured and taken as the crystalline response that would be subtracted from further results to yield the amorphous content in each sample. The three results taken for the mean result were very consistent, the standard deviation between the samples being 0.027 J/g indicating there was not extensive variation between the set of results. The samples were also corrected for weight differences so this was also discounted. The error in using this technique is due to the differences in the sorption of the vapour as particle morphologies differ for a particular sample to the average crystalline samples. All samples were sieved through a 100μm sieve to eliminate any large aggregates that may increase the error however the technique does take into account the uptake of solvent vapour for the particular sample measured.

The 0.5% w/w amorphous value shows the greatest error during extrapolation. The main reason for this large variation can be attributed to the technique of extracting the relevant data. 0.5% w/w amorphous is a very small percentage however the sorption of the water vapour from the humidifying chambers interact different to the wholly amorphous material compared to the crystalline material. The large standard deviation can be attributed to the wetting or possible increased uptake or adsorption of water compared to the crystalline material. As the average crystalline response is subtracted this causes a large variation in the measurement since the adsorption/absorption represents a significant proportion of the curve.
4. RESULTS AND DISCUSSION

4.4.9.2 Griseofulvin calibration curve method B results

Figure 4.7 - Calibration curve using data analysis of Method B for physical mixtures of quenched/crystalline griseofulvin from 0.5% to 5% w/w amorphous content.

Figure 4.7 displays the calibration curve using Method B for the same data set as used in Method A, however takes into account the desorption region when the RH switches from 95% to 0% RH. The crystalline sample, indicated on the graph as the point at 0% amorphous content displays a value of 0.060 J/g, indicating this method shows less difference to that in Method A which gave a value of 1.25 J/g for the crystalline material. Both methods show a similar relationship in the averaged data as shown in Figure 4.10, however Method B indicates considerably less difference in the variance of each data set. There is some slight curvature of the data also noticed in other work (Ramos, 2007). An explanation may be with physical mixtures as the concentration of fines on the surface of the crystalline material increase there may be a greater proportion of ethanol vapour that is retained by the fines or incorporated within the structure and not evacuated as the p/p₀ is switched from 95% to 0%. This would result in the values for increasing amounts of amorphous material present as fines on the surface to have a slightly higher enthalpic value than the true value. Further investigations are required to ascertain this possible explanation.
4. RESULTS AND DISCUSSION

4.4.9.3 Griseofulvin calibration curve comparison of Method A & B

The two methods used for the quantification of the amorphous content in griseofulvin are shown in Figure 4.8. Method A has greater error most notable at the very low amorphous content of 0.5% w/w. Method B showed a linear correlation ($R^2 = 0.95485$) when taking into account the 0.5% w/w amorphous content however when correlating from 1-5% w/w amorphous this correlation improved ($R^2 = 0.99365$). Quantification can be undertaken for samples with greater than 1% w/w amorphous content for griseofulvin. Method B was far superior to Method A as there was less variation between the measurement at each data set and the method took into account differences of each sample as the sorption and desorption for that particular sample was taken into account during measurement. The issue associated with particle morphology and using a mean crystalline response was not apparent using Method B.
4. RESULTS AND DISCUSSION

<table>
<thead>
<tr>
<th>Amorphous content</th>
<th>Method A Mean (S.D.)</th>
<th>Method B Mean (S.D.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>1.25 (0.03)</td>
<td>0.06 (0.01)</td>
</tr>
<tr>
<td>0.5</td>
<td>-0.86 (4.61)</td>
<td>0.63 (0.11)</td>
</tr>
<tr>
<td>1</td>
<td>0.58 (1.54)</td>
<td>1.44 (0.37)</td>
</tr>
<tr>
<td>2</td>
<td>5.03 (0.64)</td>
<td>4.35 (0.60)</td>
</tr>
<tr>
<td>3</td>
<td>8.67 (0.68)</td>
<td>8.23 (1.75)</td>
</tr>
<tr>
<td>4</td>
<td>10.65 (2.39)</td>
<td>11.95 (0.59)</td>
</tr>
<tr>
<td>5</td>
<td>11.39 (2.91)</td>
<td>17.08 (0.69)</td>
</tr>
</tbody>
</table>

Table 4.1 - The comparison of data analysis Method A and B to calculate the enthalpy of crystallisation for griseofulvin

With Method A there were instances when the enthalpy of crystallisation after analysis displayed a negative value such as that for 0.5% w/w in Figure 4.10. This is due to the average crystalline response having a greater exothermic peak than the physical mixtures. This was not expected as crystalline griseofulvin with amorphous fines would have a greater response than crystalline material however any small error in the small sample weight could have resulted in the differences observed. From Table 4.1 it can be seen the negative value of -0.86% w/w amorphous content was calculated using Method A. Using Method B this value with the same data set showed an amorphous content of 0.63% w/w highlighting the differences in extrapolating the data. This measurement also had the highest standard deviation between the triplicate responses with a standard deviation of 4.61 which indicates the measurement at this level is inaccurate and leads to large variation in the quantification of amorphous content.
4. RESULTS AND DISCUSSION

4.5 Budesonide Results and Discussion

4.5.1 Budesonide perfusion isothermal microcalorimetry data

Both methods were utilised in the same way to extrapolate the relevant data for budesonide using the organic solvent ethanol. Figure 4.11 shows the crystallisation of a 5% w/w physical mixture of budesonide within the RH controlled perfusion unit.

![Figure 4.9 - RH perfusion microcalorimetry plot of a 5% w/w amorphous physical mixture of quenched/crystalline budesonide mixture in a step program of 0-95-0% p/p₀ ethanol vapour to initiate crystallisation.](image)

As can be seen in Figure 4.9 the recrystallisation of budesonide is markedly different to the recrystallisation of griseofulvin in Figure 3.4. The crystallisation event is not significantly clear visually from the graph, however it can be noticed there is a substantial difference between the sorption/crystallisation portion of the curve then the desorption region, attributed to the crystallisation event. The crystallisation of the
amorphous fines is incorporated within the adsorption/absorption peak and therefore the methods of extrapolation used for griseofulvin are again repeated to construct a calibration curve for budesonide.

4.5.1.1  Budesonide calibration curve Method A results

Figure 4.10 - Calibration curve using data analysis of Method A for physical mixtures of quenched/crystalline budesonide from 0.5% to 5% w/w amorphous content.

Figure 4.10 displays the calibration curve obtained when using Method A to extract the data for low amorphous content physical mixtures. Using the average crystalline response showed a correlation with an $R^2$ of 0.97738 which shows linear correlation. The method again shows very large variations in the calculation of amorphous content % w/w. This is again due to the differences in particle morphology and the technique not being sample specific. The method does not provide an accurate method by which to examine the amorphous content of samples with small levels of disorder.
4. RESULTS AND DISCUSSION

4.5.1.2 Budesonide calibration curve Method B results

Figure 4.11 - Calibration curve using data analysis of Method B for physical mixtures of quenched/crystalline budesonide from 0.5% to 5% w/w amorphous content.

Figure 4.11 above shows the calibration curve for budesonide using Method B to extrapolate the data. The linear correlation of the data set gives a $R^2$ of 0.99671. The data sets are in good agreement and the standard deviation shows very little difference between samples. On inspection the crystalline sample, shown as the sample with 0% w/w amorphous content lies above the expected point where zero enthalpy is expected for this point. This was not found when using Method A to extrapolate the data as shown in Figure 4.10. An explanation of this where the entire data set is shifted up the y-axis seems to show an event occurring during the adsorption/absorption phase which does not occur during the desorption phase.

One explanation is that the material upon being received has a small amount of disorder present on the surface. This would not be observed when using Method A because the reference would be a partially amorphous control. Therefore the
extrapolation would remove this from the curve so it would not be seen. The presence of amorphous material present on the surface of the active may also explain the large variation seen using Method A. Since the technique was not sample specific a greater variation would be seen as the sample would have varying degrees of disorder present at the surface. Once subtracting a partially amorphous response there may be greater error in the final data.

With regards to Method B it was not initially noticed, during the crystallisation event it would be incorporated within the crystallisation of the amorphous fines since crystallisation proceeds very rapidly. With Method B there would be a standard error such as that seen using the calibration curve where the curve is shifted up, however the intercept is not at 0. Another explanation is the presence of a polymorph however standard DSC, Hyper DSC and XRPD showed no evidence of any polymorphic state change.

4.5.1.3 Budesonide calibration curve comparison of Method A & B

![Figure 4.12 - Calibration curve using data analysis of Method A and Method B for physical mixtures of quenched/crystalline budesonide from 0.5% to 5% w/w amorphous content.](image-url)
4. RESULTS AND DISCUSSION

A comparison of the two methods for budesonide is shown in Figure 4.12. There are marked differences between the two methods when correlating the amorphous content and the enthalpy of crystallisation. Taking into account an event that may be occurring and highlighted by using Method B, Method A should discount this effect however, that should not affect the slope of the graph only the placement along the y-axis. If there is an event that occurs during the initial adsorption phase Method A will be affected more greatly then Method B. Method B negates everything apart from the amorphous crystallisation and any other events that may be occurring. The event that is thought to occur with budesonide is a crystallisation event of the material as received. This would explain the large error noticed when using Method A and the intercept above 0 enthalpy for crystalline material. Table 4.2 can be used to compare the data extraction techniques, where the standard deviation of the data for Method A greater than that for Method B. The value of 1.11% w/w amorphous for the crystalline sample indicated an event occurring within the exothermic sorption section which is possible crystallisation of amorphous regions within the as received material. This may also explain the difference in the slope when using the two methods of analysis. To eliminate the effect of the possibility of amorphous material present on the surface the sample could be conditioned by exposing it to a plasticiser to successfully lower $T_g$ or induce crystallisation, possibly as an extra step in the perfusion unit prior to analysis.

<table>
<thead>
<tr>
<th>Amorphous content</th>
<th>Method A</th>
<th>Method B</th>
</tr>
</thead>
<tbody>
<tr>
<td>%</td>
<td>Mean</td>
<td>S.D.</td>
</tr>
<tr>
<td>0</td>
<td>0.00</td>
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</tr>
<tr>
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<td>5</td>
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</tr>
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</table>

Table 4.2 – Table of results for Method A and Method B for the enthalpy of crystallisation for budesonide
4. RESULTS AND DISCUSSION

4.6 INDOMETACIN RESULTS AND DISCUSSION

4.6.1 Indometacin perfusion isothermal microcalorimetry data

Calibration curves for indometacin were constructed using both methods of extrapolation. The organic solvent ethanol was used as the crystallising solvent and has been shown in previous studies (Ambarkhane 2006) to lower the $T_g$ of indometacin sufficiently to enable characterisation of the crystallisation event.

4.6.1.1 Indometacin calibration curve Method A results

![Graph showing calibration curve for Method A](image)

The data displayed in Figure 4.13 shows the relationship between the enthalpy of crystallisation and the amorphous content using Method A data analysis. As shown for the previous actives a negative response was observed for the very low amorphous content points of 0.5% w/w and 1% w/w. This shows the error is with the technique as by using Method B negative values for the enthalpy of crystallisation are not seen. The reason is due to the use of the crystalline samples as an average.
The average response used may not be fully representative of the batch therefore when a sample is measured that has the crystalline portion with a lower value then the averaged a negative value will result.

The crystallisation of the small percentage of amorphous fines does not compensate for this difference in morphology therefore it is either important to use a greater number of samples to be used as an average response or use Method B which is sample specific. The results show the difference in measurement techniques when analysing the same data in two different ways. The correlation between the enthalpy of crystallisation and amorphous content gave the $R^2$ value of 0.98115 which shows a positive correlation between the data however not accurate for very low level disorder.

4.6.1.2 Indometacin calibration curve Method B results

![Indometacin calibration curve Method B results](image)

$y = 0.00911 + 0.67201x$

$r^2=0.99634$

Figure 4.14 – Calibration curve using data analysis of Method B for physical mixtures of quenched/crystalline indometacin from 0.5% to 5% w/w amorphous content.

The data presented in Figure 4.14 for indometacin shows a method of analysis that can be used with accuracy to determine the amorphous content from 0.5% amorphous w/w. The $R^2$ of 0.99634 shows very good correlation for enthalpy of crystallisation and the amorphous content. Using the same data as that used for
Method B highlights the importance of using an accurate technique to extrapolate the relevant data.

![Calibration curve using data analysis of Method A and Method B for physical mixtures of quenched/crystalline indometacin from 0.5% to 5% w/w amorphous content.](image)

Figure 4.15 – Calibration curve using data analysis of Method A and Method B for physical mixtures of quenched/crystalline indometacin from 0.5% to 5% w/w amorphous content.

Figure 4.15 yields some interesting observations when comparing the two methods of extrapolation. The linear fit for Method A lies somewhat lower than that of Method B, with a very similar gradient. The intercept for the line of best fit for both data sets highlight the differences. Method A is associated with greater error due to the extrapolation not taking into account sample heterogeneity. This was overcome as described for all three actives using Method B of analysis. Using the desorption curve from the perfusion unit largely negates the processes occurring concurrently with crystallisation. The investigation used a very small sample size, between 50mg to 100mg which may be increased to improve the accuracy of the results. The high sensitivity of the isothermal microcalorimetry apparatus ensures accurate results may be obtained with a small sample mass.
4. RESULTS AND DISCUSSION

<table>
<thead>
<tr>
<th>Amorphous content</th>
<th>Method A</th>
<th></th>
<th>Method B</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>%</td>
<td>Mean</td>
<td>S.D.</td>
<td>Mean</td>
<td>S.D.</td>
</tr>
<tr>
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<td>0.02</td>
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<td>0.06</td>
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<td>0.06</td>
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</tr>
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<td>2.68</td>
<td>0.04</td>
<td>3.27</td>
<td>0.12</td>
</tr>
</tbody>
</table>

Table 4.3 – Table of results for Method A and Method B for the enthalpy of crystallisation for indometacin

Table 4.3 shows the values for the two techniques for the determination of the relationship between the enthalpy of crystallisation and the amorphous content. The negative values for 0, 0.5% and 1% w/w amorphous content of -0.21, -1.22 and -0.21 respectively show the limitations of Method A. The standard deviation using Method B on the same data set establishes a more accurate method of analysis.

4.7 Micronisation study

For the purpose of milling only indometacin and griseofulvin were taken forward. Budesonide posed health and safety issues, due to the propagation of dust using the air jet mill and the rotary mill. Consideration was taken however the active was not used for further experiments.

The actives were milled using three different milling techniques. For air jet milling a range of pressures within the inlet and outlet were varied. The Fritsch rotary mill consisted of a chamber with porcelain balls rotating at 300rpm with the time of milling varied. The high vibration ball mill consisted of a small chamber with an agate ball that used a multi-directional movement to cause size reduction. The increase in the time milled would be investigated as a function of amorphous content. A further description of the methods involved is detailed in Section 3.8. Using Method B the approximate amorphous content of the samples was calculated.
from the enthalpy of crystallisation. Preliminary milling on the air jet mill yielded results to indicate the amorphous content was below 10% amorphous w/w.

4.7.1 **Griseofulvin micronised data**

4.7.1.1 **Air jet milled griseofulvin results**

![Graph showing amorphous content](image.png)

**Figure 4.16** – The amorphous content as calculated using Method B of analysis from triplicate batches of milled griseofulvin in the air jet microniser at the respective levels of pressure (psi).

Figure 4.16 graphically represents the change in amorphous content of griseofulvin post-milling. As the inlet and outlet pressures are increased there is an increase in the amorphous content, particularly at the pressure of 80/80 psi which exhibits approximately 2% amorphous content w/w. DSC and x-ray diffraction traces did not conclude any change in the properties of griseofulvin. It can also be noted that as the mill pressure is increased there is also an increase in the variability of the amorphous content noted by the increase in the variation between the data sets. The active that was milled was collected from the chamber within the mill, however at higher pressures it was observed less active accumulated within the valves and a larger...
4. RESULTS AND DISCUSSION

proportion collected into the chamber. This may be an explanation of the increase in the variability in the amorphous content at mill pressure of 80/80 psi.

4.7.1.2 Fritsch Rotary ball mill griseofulvin results

![Graph showing amorphous content over time](image)

Figure 4.17 – The amorphous content as calculated using Method B of analysis from triplicate batches of milled griseofulvin using the Fritsch ball mill at 1, 5, 10 and 24 hours mill time at 300 rpm.

Another milling technique was employed to understand the change in amorphous content as the milling time was increased. Variables such as load volume, speed, number of balls used remained constant however the time of milling was adjusted. The results after using Method B to extrapolate the data can be seen in Figure 4.17. Variation in the mill is larger than that observed then the air jet mill. This may be technique specific since the ball mill impacts the active on the walls of the container. Mixing of the entire contents within the milling chamber was undertaken however this may not have been sufficient to enable homogenous milling. The air jet mill operates by the impaction of two particles colliding to initiate fracture, and once a specific size is reached enters the collecting chamber. From Figure 4.17 it can also be observed that there was approximately 10% amorphous content produced with 24
4. RESULTS AND DISCUSSION

hours milling and this was detected on X-ray diffraction patterns with the appearance of a halo shape and a crystallisation endotherm when analysed on the DSC.

4.7.1.3 High vibration ball mill griseofulvin results

![Graph showing amorphous content over time](image)

Figure 4.18 – The amorphous content as calculated using Method B of analysis from triplicate batches of milled griseofulvin using the vibration ball mill at 5, 10, 15 and 30 minutes.

The high vibration ball mill used a multi-directional force to mill the active within an agate chamber using an agate ball to initiate fracture of particles. There was considerably less variation then compared to the Fritsch ball mill which may be due to a decrease in the amount of active that impacts upon the chamber wall. In the Fritsch mill the high revolutions causes powder to spread on the surface of the container with successive impaction impeding the milling of the entire active. This is why mixing of the active is important otherwise there is a solid build up of active on the chamber walls. In the high vibration ball mill this impaction on the surfaces still occurs but to a lesser extent. Due to the multi-directional force there is less impaction on any one side of the surface. Figure 4.18 also shows that an increase in milling time increases the amorphous content in a linear relationship. The rate increases initially from 5 to 15 minutes and then decreases from 15 to 30 minutes which may indicate the process of new amorphous sites slows.
**Micronised griseofulvin amorphous data**

<table>
<thead>
<tr>
<th>Pressure (psi/psi)</th>
<th>Air jet Mill Amorphous content % (S.D.)</th>
<th>Fritsche Mill Amorphous content % (S.D.)</th>
<th>Ball Mill Amorphous content % (S.D.)</th>
<th>Time milled (minutes)</th>
</tr>
</thead>
<tbody>
<tr>
<td>20/20</td>
<td>0.10 (0.02)</td>
<td>3.46 (1.18)</td>
<td>0.54 (0.03)</td>
<td></td>
</tr>
<tr>
<td>40/40</td>
<td>0.40 (0.07)</td>
<td>3.63 (0.53)</td>
<td>1.04 (0.02)</td>
<td></td>
</tr>
<tr>
<td>60/60</td>
<td>0.56 (0.04)</td>
<td>5.96 (0.69)</td>
<td>2.26 (0.29)</td>
<td></td>
</tr>
<tr>
<td>80/80</td>
<td>2.03 (0.35)</td>
<td>10.49 (1.03)</td>
<td>2.90 (0.10)</td>
<td></td>
</tr>
</tbody>
</table>

Table 4.4 – The amorphous content calculated using Method B for micronised griseofulvin using three milling techniques. Results shown are an average of three batches micronised for each technique.

Table 4.4 shows the differences in the amorphous content induced by milling for the three techniques. The data presented indicate air jet milling results in the least amount of amorphous griseofulvin, with a maximum amorphous content of 2.03% w/w using a mill pressure of 80/80 psi. High vibration ball milling results in a maximum amorphous content of 2.90% w/w after 30 minutes of milling however the rotary ball mill after 24 hours indicates a significant of the bulk is amorphous with 10.49% w/w amorphous. This measured value is not within the limits of the calibration curve constructed however it is assumed the relationship measured from 0.5% to 5% w/w amorphous content as physical mixtures is linear and a direct correlation can be made.
4. RESULTS AND DISCUSSION

4.7.2 Indomethacin micronised data

4.7.2.1 Air jet milled indomethacin results

The air jet milled data presented in Figure 4.19 for indomethacin indicates a gradual increase in the amorphous content as the inlet and outlet pressure is increased. Another point to note is the increase in the variation in the data sets as the mill pressure is increased. Comparing the data from Table 4.4 the pressure of 20/20 psi resulted in 0.42% w/w amorphous content compared to 1.73% w/w amorphous content using 80/80 psi. As the data suggested for griseofulvin the variability for the higher milling pressure led to a greater standard deviation. The active milled batch at 20/20 psi and 40/40 psi had a standard deviation of 0.05 and 0.09% respectively. For the higher pressure, the standard deviation was 0.29% and 0.3% for 60/60 psi and 80/80 psi respectively. This shows a large increase in the variation within one batch of material milled.
4. RESULTS AND DISCUSSION

4.7.2.2 Fritsch rotary ball mill indometacin results

![Graph showing amorphous content over time](image)

**Figure 4.20** - The amorphous content as calculated using Method B of analysis from triplicate batches of milled indometacin using the Fritsch ball mill at 1, 5, 10 and 24 hours mill time at 300 rpm.

The amorphous content calculated using Method B for the Fritsch ball mill shows significant changes as shown in Figure 4.20. The Fritsch mill induces amorphous character in indometacin up to a maximum of 17.28% w/w which is a significant proportion of the material. This particular time also gave the largest variation in the results with the standard deviation being 5.20% as shown in Figure 4.21 and Table 4.5. The variation can be explained by the technique and the duration of milling. As the indometacin was milled for that length of time there was impaction of the material on the sides of the container. The agate balls within the container would continuously cause attrition of the particles at the surface of the container, however powder that is compacted at the lower layers would have less amorphous character than particles present at the upper layers. Therefore a greater variation would be observed once the powder was sieved and mixed as one batch of milled active. The effect of the variation due to impaction increased with time as shown in Figure 4.21.
4.7.2.3 High vibration ball milled indomethacin results

The high vibration ball mill used a small sample size and a multi-directional force to impart stress on the active. The results show (Figure 4.21, Table 4.5) there is an increase in the amorphous content as the milling time increases up to a maximum of 2.46% w/w amorphous content as extrapolated using Method B. As for previous techniques there is an increase in the standard deviation from 0.22% at 5 minutes milling to 0.5% at 30 minutes milling. This is again due to a similar observation as that made in the Fritsch mill, where the solid would aggregate on the sides on the container. This would result in the top layers of the active undergoing attrition with the bottom layers less disordered. This would increase the variability at time increases due to increase. At regular intervals of 10 minutes the sample would be mixed however impaction still occurred using both techniques.
4. RESULTS AND DISCUSSION

### Indometacin micronised data

<table>
<thead>
<tr>
<th>Pressure (psi/psi)</th>
<th>Air jet Mill</th>
<th>Fritsch Mill</th>
<th>Ball Mill</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Amorphous content % (S.D.)</td>
<td>Amorphous content % (S.D.)</td>
<td>Amorphous content % (S.D.)</td>
</tr>
<tr>
<td><strong>Time milled</strong></td>
<td><strong>Time milled</strong></td>
<td><strong>Time milled</strong></td>
<td><strong>Time milled</strong></td>
</tr>
<tr>
<td></td>
<td>(hours)</td>
<td>(hours)</td>
<td>(minutes)</td>
</tr>
<tr>
<td>20/20</td>
<td>0.42 (0.05)</td>
<td>1.48 (0.48)</td>
<td>0.63 (0.22)</td>
</tr>
<tr>
<td>40/40</td>
<td>0.59 (0.09)</td>
<td>6.02 (0.45)</td>
<td>0.94 (0.10)</td>
</tr>
<tr>
<td>60/60</td>
<td>1.08 (0.29)</td>
<td>11.83 (1.71)</td>
<td>1.36 (0.33)</td>
</tr>
<tr>
<td>80/80</td>
<td>1.73 (0.30)</td>
<td>17.28 (5.20)</td>
<td>2.46 (0.50)</td>
</tr>
</tbody>
</table>

Table 4.5 - Amorphous content determined using Method B for micronised indometacin using three milling techniques
CHAPTER FIVE

THE INVESTIGATION OF THE SURFACE CHARACTERISTICS OF HYDROPHOBIC ACTIVES POST MICRONISATION AND DURING STORAGE AT A RANGE OF RELATIVE HUMIDITIES
5. RESULTS AND DISCUSSION

5.1 INTRODUCTION

Many pharmaceutical processes depend upon the surface characteristics of the constituents and the interaction of forces involved during formulation. It is therefore highly important to understand the surface mediated reactions and the changes that may occur to the surface during development. Processing can often induce transformations within an active that can lead to changes in the crystal habit, polymorphic form or the presence of unstable amorphous regions. Traditionally surface properties have been measured using the contact angle technique however the techniques have several limitations most notably for powdered systems (Buckton et al., 1995a).

Inverse Gas Phase Chromatography (IGC) is a dynamic vapour sorption technique that investigates the surface properties of the sample using the interaction of vapour probes with known characteristics. The technique is non-destructive, non-invasive and requires minimal sample for measurement. The affinity of the vapour probe to the surface determines the retention times with the response measured by detectors in the instrument. The behaviour of the specific probes is used to determine the surface characteristics such as the powder surface energies and acid, base and polar functionality of surfaces.

One of the earliest studies of IGC research was measuring the adsorption of water at zero and finite surface coverage of cyclosporin A (Djordjevic et al., 1992) and the adsorption of organic vapours onto different microcrystalline cellulosics (Ticehurst et al., 1993). The technique was then used to assess the difference and highlighted batch to batch variability in salbutamol sulphate (Ticehurst et al., 1994) and α-lactose (Ticehurst et al., 1996) where other techniques showed the batches to be indistinguishable by standard analytical techniques. The effect of milling on the surface properties of \textit{dl}-propranolol was investigated (York et al., 1998) which indicated the surface of the active became more energetic and electron donating with milling.

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The use of IGC in quantifying surface energetic data and relating this to secondary processing such as powder flow showed important progress (Feeley et al., 1998). Differences in the surface energy of salbutamol sulphate after micronisation were related to the powder flow and compared to unmicronised batches and gave an important insight into the variability that can go undetected in pharmaceutical actives. This highlighted the importance of surface characterisation and understanding the behaviour of pharmaceutical actives during processing.

More recently the effect of milling and a change in humidity on the surface of cefditoren pivoxil has been studied (Ohta & Buckton, 2004a). The application of the technique has increased in complexity at measuring and predicting performance within a system. Further work (Ohta & Buckton, 2004b) has showed how incompatibility between polymers and cefditoren pivoxil could be explained by showing the change in acidic and basic contributions of the surface.

The investigation into the difference in polymorphs for xemilofiban showed differences in the non-polar interactions (Butler & Mackin, Application note 207). The technique also showed how amorphous regions present on Polymorph A were analysed using the technique helping to evaluate the behaviour of the surface and crystallisation using vapour probes. Further developments have shown the technique capable of measuring the glass transition temperature of a hydrophobic active, indometacin using the retention characteristics of decane (Buckton et al., 2004).
5. RESULTS AND DISCUSSION

5.2 AIMS

The aim of the chapter was to investigate the surface energetic properties of the two hydrophobic actives griseofulvin and indometacin to understand the change in surface energetic post micronisation. The non-polar interactions and polar interactions were studied to understand the surface energy changes that occur during high energy milling techniques to evaluate the degree of change and the comparison between different techniques of milling.

Studies were undertaken to assess the change in the surface of the compound post micronisation during storage at a range of humidities from 0% and 11.3% RH and 57.5% to 75% RH to simulate high levels of humidity. Batches were tested for surface energetic data including non-polar interactions and polar interactions to determine the extent of acidic or basic change at the surface during storage.

The investigations into the enthalpic change for the two hydrophobic actives griseofulvin and indometacin were undertaken to investigate the recrystallisation of both actives to correlate to surface energetic data. Samples would be investigated for the change in amorphous content using the method outlined in Chapter 4 for the determination of low amorphous content for micronised hydrophobic actives.
5.3 EXPERIMENTAL METHOD

5.3.1 Introduction

The principals of IGC are very simple, being the opposite to a conventional gas chromatographic experiment. An empty column is uniformly packed with a powdered sample. A carrier gas set at a specified flow rate delivers an injection, typically a gas probe and the retention behaviour as it passes through the column is measured by a detector. There are two types of IGC measurement, a frontal technique where the probe molecule is added to a continuous flow of carrier gas and the chromatograph shows a breakthrough curve. In an alternative approach, the pulse technique carries a certain concentration of vapour probe by the carrier gas through the sample. Adsorption and desorption of the probe results in a peak of the probe and the characteristics of this retention time are used to calculate the surface energy of the sample.

The time taken for the probes to elute from the column or the retention times \( t_r \) can be used to calculate the dispersive component \( (\gamma_s^d) \) and polar acid base parameters \( (K_A \) and \( K_D) \) of the powders using a series of thermodynamic equations.

The probes that are passed through the column will interact with the sample (stationary phase) in different ways. The molecular adsorption will result in the elution of the probe at different times which is referred to as the retention time \( t_r \).

The carrier gas for the probes is either helium or nitrogen. There is a delay period that must be taken into account and using an inert probe i.e. methane a reference elution time can be calculated to account for this delay \( t_0 \). The net retention time \( t_n \) is calculated by:

\[
t_n = t_r - t_0 \quad \text{Equation 5.1}
\]

To take into account the packing of the column and flow rate the net retention volume is calculated by applying a correction factor for the change in flow during
the pressure drop experienced when the probe is injected. The net retention volume \( V_N \) is calculated as follows:

\[
V_N = JF (t_r - t_o) \quad \text{Equation 5.2}
\]

\( t_r \) is the retention time of the probe
\( t_o \) is the retention time of methane
\( F \) is the flow rate (ml/min) of the carrier gas
\( J \) is the compression factor for the pressure drop due to the compressibility of the gas over the column and is calculated by Equation 5.3.

\[
J = \frac{3 \left[ (P_i/P_o)^2 - 1 \right]}{2 \left[ (P_i/P_o)^3 - 1 \right]} \quad \text{Equation 5.3}
\]

\( P_i \) is the pressure of the inlet column
\( P_o \) is the pressure of the outlet column

The free energy of adsorption \( (\Delta G_A^0) \) is composed of polar and non-polar interactions for a gaseous probe, under isothermal conditions by Equation 5.4,

\[
\Delta G_A^0 = \Delta G_A^D + \Delta G_A^S \quad \text{Equation 5.4}
\]

\( \Delta G_A^D \) is the non-polar free energy of adsorption
\( \Delta G_A^S \) is the polar or specific free energy of adsorption

The free energy of adsorption \( (\Delta G_A^0) \) for a gaseous probe can be linked to \( V_N \) by a series of calculations to provide Equation 5.5.

\[
\Delta G_A^0 = -RT \ln (V_N P_{se}/\pi w Sa) \quad \text{Equation 5.5}
\]

\( R \) is the gas constant
\( T \) is the temperature of the column (K)
5. RESULTS AND DISCUSSION

$P_{sg}$ is the adsorbate vapour pressure in the gaseous standard state ($1.013 \times 10^5$ Pa)

$\pi$ is the standard surface pressure ($3.37 \times 10^4$ N/m$^2$) (De Boer 1953)

$w$ is the weight of the adsorbant in the column

$S_0$ is the surface area of the adsorbent (m$^2$/g)

Since $S_0$, $P_{sg}$ and $w$ are all constant these terms can be reduced to the constant $K'$ (Schultz et al., 1987) Equation 5.5 is thus reduced to:

$$\Delta G_A^0 = -RT \ln V_N + K'$$

Equation 5.6

5.3.2 Dispersive Surface Energy

The free energy of adsorption of an apolar probe can be related to the work of adhesion by Equation 5.7:

$$\Delta G_A^0 = N_A \cdot a \cdot W_A$$

Equation 5.7

$a$ is the cross sectional area of the adsorbate and $N_A$ the Avogadro constant.

According to Fowkes 1964, $W_A$ can be split into two terms as in Equation 5.8:

$$W_A = W_A^D + W_A^S$$

Equation 5.8

With $W_A^D$ denoting the van der Waals forces and $W_A^S$ the specific, mainly polar interactions. Subsequently the retention volume is a measure for both components. In the case of dispersive interactions $W_A$ is given by Equation 5.9

$$W_A = 2(\gamma_S^D \cdot \gamma_L^D)^{1/2}$$

Equation 5.9

With $\gamma_S^D$ and $\gamma_L^D$ as the surface tension of the adsorbent and the adsorbate. To calculate the dispersive surface energy the method of Schultz et al (1987) uses a plot of $\Delta G$ versus $a(\gamma_L^D)^{1/2}$ for a series of homologous alkanes.
A plot of the free energy of adsorption (RT\ln V_N) as a function of the alkane surface area and the dispersive component of the alkane surface energy a(\gamma_L^D)\frac{1}{2} a straight line is obtained. The dispersive component of the solid (\gamma_S^D) can then be obtained by the slope of the line.

![Surface and Free Energy Plot](image)

Figure 5.1 – Illustration of the free energy of adsorption of alkane and polar probes. Adapted from Theilman et al. Application note 221

By combining the equations 5.7, 5.8 and 5.9 the formula becomes:

\[ RT\ln V_N = 2N (\gamma_S^D)^{\frac{1}{2}}a(\gamma_L^D)^{\frac{1}{2}} = K' \]  
Equation 5.10

A plot of the free energy of adsorption versus the dispersion component of the alkanes provides a straight line (Figure 5.1). From the slope of the line the dispersive component of the solid line can be calculated, 2N (\gamma_S^D)\frac{1}{2}. To create such plot values of \('a' and \gamma_L^D of the alkanes are required. These values were obtained from literature (Schultz et al., 1987; Nardin and Papirer, 1990).
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5.3.3 Polar Interactions

Interactions of the polar probes with a solid involve both Van der Waals forces (dispersive component) and polar interactions (specific or acid-base interactions). As a result the interactions of a polar probe would be expected to provide higher energies of adsorption than non-polar probes. The distance between the polar probe and the alkane line (Figure 5.1) is the specific free energy of adsorption ($\Delta G^{\text{SP}}_A$) and the distance to the x-axis is termed the total free energy of adsorption ($\Delta G^0_A$).

<table>
<thead>
<tr>
<th>Vapour probe</th>
<th>Molecular Area ($\text{m}^2 \times 10'$)</th>
<th>$\gamma^\text{p}_{\text{l}}$ (mJ/m$^2$)</th>
<th>AN* (kJ/mol)</th>
<th>DN (kJ/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>n-Decane</td>
<td>7.5</td>
<td>23.4</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>n-Nonane</td>
<td>6.9</td>
<td>22.7</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>n-Octane</td>
<td>6.3</td>
<td>21.3</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>n-Heptane</td>
<td>5.7</td>
<td>20.3</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>n-Hexane</td>
<td>5.2</td>
<td>18.4</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Acetone</td>
<td>3.4</td>
<td>16.5</td>
<td>10.460</td>
<td>71.128</td>
</tr>
<tr>
<td>Chloroform</td>
<td>4.4</td>
<td>25.0</td>
<td>22.5936</td>
<td>0</td>
</tr>
<tr>
<td>Ethanol</td>
<td>3.3</td>
<td>19.6</td>
<td>43.0952</td>
<td>83.680</td>
</tr>
<tr>
<td>Ethyl Acetate</td>
<td>3.3</td>
<td>19.6</td>
<td>6.276</td>
<td>71.5464</td>
</tr>
</tbody>
</table>

Table 5.1 – Table of vapour probe characteristics used in IGC experiment

Having determined the $\Delta G^{\text{SP}}_A$, the acid-base properties of the surface can be calculated from each probe. Gutmann (1978) suggested that the heat of the acid-base interaction of each probe is related to a Gutmann acceptor and donor number; the acceptor number (AN) being the acid component and the donor component (DN) related to the basic component as essentially they are Lewis acid-base interactions. Also the Gutmann approach allows the amphoteric nature of liquid such as acetone to be accounted for. Gutmann suggested that strong interactions only develop between an acid and a base. Therefore materials of a similar nature will create an almost zero specific interaction. Some probes poses high AN values due to
5. RESULTS AND DISCUSSION

contributions from Van der Waals forces, thus AN* was created (Riddle and Fowkes, 1990). This value only takes into account of the polar interactions. The values and other properties of the solvents have been listed in Table 5.1.

The acid-base parameters \( (K_A \text{ and } K_D) \) can then be used to describe the specific free energy of adsorption:

\[
-\Delta G_A^{SP} = K_A D N + K_D A N^* \quad \text{Equation 5.11}
\]

A plot of \( \Delta G_A^{SP}/AN^* \) against \( DN/AN^* \) for various probes will provide values for the \( K_A \) and \( K_D \) nature for the surface of the compound.

The acid-base characteristics can also be calculated using other physical properties of the injected probes. Alternate properties can be plotted against \( RT\ln V_N \) to determine the free energy of adsorption values. The conventional method plots the molecular area of each probe \( 'a(\gamma DL)^{1/2}(m^2.(J/m^2)^{1/2})' \) against \( RT\ln VN \) (Schultz et al., 1987; Nardin & Papier, 1990).

5.3.4 Methodology

It was highly important to ensure the experimental methodology was undertaken in a precise manner to minimise issues as such poor gas flow, changes in surface energy from samples of the same batch and changes during the experiment which can often be attributed to poor sample packing. In order to study the surface energetics the actives were packed into silanised glass columns as described in Section 3.3.3. The glass columns used had an internal diameter of 4mm and were 30mm in length.

Prior to measurement columns were conditioned in the oven for 2 hours at the appropriate temperature and humidity. Conditioning ensured there were minimal contaminants present within the column. Any weakly adsorbed particles present on the surface would be removed via the carrier gas before the experiment would be started. Once the experiment was initiated the probes would be injected in the specified sequence at an infinite dilution (0.04% v/v) At infinite dilution, the probe
molecules have minimal lateral interactions with each other and therefore maximising the interaction of the probe with the material surface.

The probes that were used were HPLC grade (>99.99% purity) and a list is shown in Table 6.1. Methane gas (BOC) was used as the reference probe with Helium (BOC) used as the carrier gas. The probes were held at 303K and the columns were held at 298K. The relative humidity was kept at 0% RH. The helium gas flow was variable throughout the experiment to ensure a good balance between the elution speed, shape of solute peak and pressure drop along the column. Results were obtained from a triplicate response.

5.3.5 Calibration

The system would be regularly maintained and an inorganic reference material namely α-alumina was used as a system suitability test in the IGC. This would ensure the dispersive surface energies were within the expected range of between 30-35 mJ/m². Approximately 1g of α-alumminia was packed into a silanised column and conditioned at 25°C and conditioned for 2 hours at 0% RH prior to analysis with a flow rate of 10 sccm. Any large deviation from the expected dispersive surface energy would require maintenance from the manufacturer.
5.4 Results and Discussion

5.4.1 Surface energetics of griseofulvin

Initial observations were undertaken on griseofulvin to compare the non-micronised active to the micronised active. The batches that were studied were the batches taken forward towards granulation described in Chapter 6.

5.4.1.1 Dispersive surface energy of griseofulvin

The dispersive surface energy of griseofulvin was determined by injecting five non-polar probes to measure the free energy of adsorption as a function of the non-polar dispersion component. Initially micronised griseofulvin was studied under ambient storage conditions. Later studies controlled the RH and temperature to study the effect of the variables on the powder properties during storage. The non-polar surface energies for micronised and stored griseofulvin are presented in Figure 5.2.

![Griseofulvin Dispersive Energy](image)

**Figure 5.2** – Bar chart of the non-polar dispersive component for griseofulvin and the change post-micronisation during ambient storage.
5. RESULTS AND DISCUSSION

The five non-polar probes used to determine the dispersive surface energy were eluted within the time frame of the experiment with a $R^2$ value at least 0.99 for all batches. Samples were tested using DSC analysis and the perfusion isothermal microcalorimeter to ensure the batch was crystalline noted by an absence of any recrystallisation exotherm. Micronisation yielded the batch with the highest non-polar value (61.6 mJ/m$^2$) with a significant difference to that of the non-micronised batch. This is largely due to the effect micronisation has on the surface of the active. The high energy process initiates fracture along the crystal planes and increases the surface area of the active. The process also creates more energetic areas on the surface of the active. The batch also undergoes amorphous change to the crystalline material as highlighted in Section 4.7.

Previous studies have shown that there is an increase in the apolar component of a partially amorphous material then its relative crystalline counterpart (Newell et al., 2001a & 2001b). During storage there is a gradual decrease in the non-polar component most likely due to relaxation and recovery of the material. The decrease has not reached levels of the non-micronised batch in the time frame of the study however and aged material shows a significant difference with respect to non-polar interactions (ANOVA, $p$-value<0.05). A comparison of the difference in the dispersive component due to the use of various techniques of micronising the active is represented in Figure 5.3.
5. RESULTS AND DISCUSSION

Griseofulvin Dispersive Energy

![Bar chart comparing the non-polar dispersive component of griseofulvin as received and post micronisation using three different milling techniques](image)

The greatest change in non-polar interactions occurred in the batch micronised using the Fritsch ball mill with a significant change from 41.3 to 61.6 mJ/m². This technique also attributed to the greatest change in amorphous content as determined in Chapter 4. Previous work (Buckton et al., 1999) has shown the relation between water sorption and surface energy measurement of tray dried saquinavir with differences attributed in processing of the powder. The surface of the saquinavir was present in a higher unstable state prior to processing which attributed to variability in the uptake of water using isothermal microcalorimetry. The results in this study indicate a similar observation whereby the unstable partially amorphous surface of griseofulvin is attributed to a higher surface energetic state. As the process of the micronisation technique is intensified there is also an increase in both the amorphous content and non-polar surface interaction. One point where this relationship did not validate was the result for the air jet milled sample. There was a decrease from 41.3mJ/m² to 36.6mJ/m² which was an unexpected decrease in the dispersive surface energy. Air jet milling was undertaken at a pressure of 60/60 psi and this yielded an amorphous content of 0.56% w/w (0.04) (Section 4.7.1.1). An earlier study (York et al., 1998) investigated the air jet milling of D,L-propranolol and the surface
energetic changes of different batches. As the median size decreased there was an initial increase until a plateau was reached where at a critical point the surface energy decreased. Interestingly the technique used was similar to the spiral jet mill used in this study and the observation was attributed to the method of fracture of specific planes using molecular modelling to determine the surfaces that were exposed. The study showed how size reduction attributed to attrition as opposed to fracture of crystal planes can expose a surface with the same functional groups and therefore the dispersive component may remain constant or decrease. Although a similar study was not undertaken in this instance the results indicate how different milling techniques can cause differences in the surface properties of the active. Air jet milling primarily uses an intensive attrition process to achieve size reduction whereas ball milling generally initiates fracture through mechanical breakage of particles exposing new surfaces.

5.4.1.2 Determination of the acid character of griseofulvin post milling

The acid/base character of griseofulvin was investigated to determine the changes in the polar component at the surface post micronisation. The acid/base character could be further used to understand the surface properties of griseofulvin. One of the polar probes used in the experiment did not elute with a Gaussian peak as there was a very strong interaction with the partially amorphous griseofulvin and has observed in previous work (Ticehurst et al., 1996).
5. RESULTS AND DISCUSSION

Griseofulvin - $K_a$

<table>
<thead>
<tr>
<th>Method</th>
<th>$K_a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Griseofulvin - Non-Micronised</td>
<td>104.3</td>
</tr>
<tr>
<td>Griseofulvin - Air jet mill</td>
<td>101.1</td>
</tr>
<tr>
<td>Griseofulvin - Vibration Ball Mill</td>
<td>125.3</td>
</tr>
<tr>
<td>Griseofulvin - Fritsch Ball Mill</td>
<td>130.9</td>
</tr>
</tbody>
</table>

Figure 5.4 - Bar chart of the differences in acid character for griseofulvin as received and post micronisation using three different milling techniques.

The change in the acid character of griseofulvin was investigated and is highlighted using a comparison of $K_a$ values in Figure 5.4. There is a notable increase in the $K_a$ values post milling using the ball milling techniques, indicating an increase in the acidic interactions post micronisation. The surface becomes more electron donating as a result of the greater polar acidic components available for interaction. Air jet milled griseofulvin actually shows a decrease in $K_a$ from the non-micronised batch compared to the vibration ball mill and the Fritsch ball mill that indicates significant increases in the $K_a$. The decrease in air jet milling may be attributed to different functional groups exposed at the surface compared to ball milling.
5. RESULTS AND DISCUSSION

5.4.1.3 Determination of basic nature of griseofulvin post milling

Figure 5.5 – Bar chart of the differences in basic character for griseofulvin as received and post micronisation using three different milling techniques.

Figure 5.5 displays the $K_D$ values for griseofulvin that were calculated by extrapolating the y-axis intercept for the polar probes. The values for the non-micronised batch and Fritsch ball mill show a negative value of -10 and -19.2 respectively. Negative values for $K_D$ are not theoretically possible so the method by which $K_D$ is calculated is not an absolute method of analysis.

The interaction of the polar probes related to the surface energy can be graphically represented with $\Delta G_A^{SP}/AN^*$ as a function of $DN/AN^*$ (Figure 5.6). The $R^2 = 0.958$ shows the relationship between then points results in the line of best fit intersecting the y-axis at a value below 0 which is the value used to measure $K_D$. This is the reason $K_D$ can appear to be negative as the calculation takes the intercept value as the true value. In this instance it must be assumed the interaction is negligible, further comparisons using the $K_D$ cannot be made for results that have negative values.
5. RESULTS AND DISCUSSION

Figure 5.6 — Graph of DN/AN$^*$ versus ΔGASP/AN$^*$ for non-micronised griseofulvin.

5.4.1.4 Stability study results

Griseofulvin was milled using the Fritsch Ball mill and stored within 0%, 11.3%, 57.5% and 75% RH using saturated salt solutions and tested for surface energetic data over a period of 7, 14 and 28 days. Samples were tested to show the difference in storage under condition of low and high humidity to determine whether any differences could be noted in surface energetic data which would then be correlated to granulation characteristics in Chapter 6.
5. RESULTS AND DISCUSSION

5.4.1.5 Dispersive surface energetic data for milled griseofulvin during storage

![Graph](image)

Figure 5.7 – Comparison of the dispersive surface energy for griseofulvin batches milled with subsequent storage under respective RH with time in days.

The non-polar interactions for micronised griseofulvin and stored under a range of RH environments from 0 to 75% over time are displayed in Figure 5.7. There is a statistical difference (ANOVA, p-value <0.05) between the lower RH storage conditions of 0% and 11.3% RH and the higher RH storage conditions of 57.5% and 75% RH for the entire time investigated. The investigations were undertaken on one micronised batch packed into a column and stored under the respective environment. This ensured sample differences and powder packing issues were negligible as one packed column was used for all experiments. The data may be analysed in one of two ways, whether in terms of an increase in RH or in terms of an increase in duration of storage. Initially if taking into account each RH level there is no significant difference at 0% RH and 11.3% RH over the time range studied (ANOVA, p-value >0.05). This shows the surface of the active varies very little during a period of 28 days after micronisation which yielded 61.6 mJ/m² for the surface energy. However the high RH environments showed significant differences to the micronised batch and specifically at 75% RH there is an indication of a
5. RESULTS AND DISCUSSION

general decrease in the non-polar interactions. A peak of 67.8 mJ/m\(^2\) is reached at 7 days storage under 75% RH which decreases to 64.4 mJ/m\(^2\) after 28 days post micronisation showing signs of recovery. At 57.5% RH there is no significant relationship that can be deduced from the data. There was an initial increase to 67.8 mJ/m\(^2\) followed by a sharp decrease in the measured values to 63.5 mJ/m\(^2\) with a return to a higher value of 67.4 mJ/m\(^2\). The data shows a more valid relationship as the data was analysed with a focus on the particular day as a function of relative humidity. This represents the data well and shows a general increase at each particular time point studied as the relative humidity increases. For all four storage humidity environments there is an increase in the non-polar interactions with respect to that particular day investigated. Earlier work (Newell et al., 2001a) showed how the surface energy varied with a change in the relative humidity for lactose where an increase in RH led to a decrease in surface energy.

Previous work by Sunkerset et al., (2001) studied the changes in the surface energy characteristics for pharmaceutical materials under conditions of dry (0% RH) and ambient (40-50%RH) humidity. The increase in humidity resulted in the non-polar interactions to either remain constant or decrease. It was also shown that the humidity had an effect on the interaction of the polar probes and found as the humidity increase in some cases there was an increased interaction at the surface. Using molecular modelling techniques it was possible to study the preferred interaction of water and concluded that the water molecules would compete for the same sites as that for the probe molecules.

This was also observed by an earlier study by Ballard et al., (1997) who investigated the influence of water on the retention of organic probes on clay. It was concluded in this study the decreases in \(\gamma^D_S\) was due to the water shielding the high energy interaction sites of the polar probes at the surface. It is possible that the adsorbed water may have been capable of altering the initial charge at sites where probes would preferentially interact to explain the increases observed which was proposed by Sunkersett (2001) for materials that showed increases in the surface energy. Another reason for the increase could be a change in the surface area of the active over time. It was observed the surface of budesonide showed an increase in the surface area of up to 22% w/w possibly due to post-micronisation stress relief by
5. RESULTS AND DISCUSSION

intraparticle crack formation, crack propagation with time, and particle fracture (Joshi et al., 2002). There is the possibility of this however further tests would be needed to validate this theory.

5.4.1.6 Determination of acid character of milled griseofulvin during storage

The polar interactions of griseofulvin were investigated as the batches of micronised active were stored under the conditions mentioned previously. The $K_A$ and $K_D$ values were measured using the Gutmann method and provided a relative means of comparing the basic and acidic interactions that occur at the surface of the active.

![Figure 5.8](image-url) - Comparison of the acidic component for griseofulvin batches micronised with subsequent storage under respective RH with time in days.

The $K_A$ values for the micronised griseofulvin stored under 0%, 11.3%, 57.5% and 75% RH over a duration of 7, 14 and 28 days can be compared in Figure 5.8. On initial inspection it seems the data follows the relationship of that observed with the non polar interactions and to some extent there are similar patterns. At higher RH storage conditions there is an increase in the acidic component compared to the
5. RESULTS AND DISCUSSION

samples held at a lower RH. At 0% RH there is a decrease in the $K_A$ values over time as well as that observed for 75% RH. The decrease in the 0% RH is statistically insignificant (ANOVA, $p$-value >0.05) however the decrease in the 75% RH is statistically significant, decreasing from 140.8 arbitrary units to 135.7 arbitrary units after 28 days. There is a small increase for the batch held at 11.3% RH and the active held at 57.5% RH shows a similar observation to that recorded for the dispersive surface energy with an initial decrease of 141.2 arbitrary units to 136.2 arbitrary units followed by a large increase to 141.4 arbitrary units.

Again when comparing the actual days there is a general increase at each specified day as the relative humidity is increased. There is evidence of a plateau reached at 75% held at 14 days with a decrease observed after 28 days storage. The results indicate the surface becomes more acidic and has a higher surface free energy as griseofulvin is stored at elevated humidity.

5.4.1.7 Determination of basic character of milled griseofulvin during storage

![Figure 5.9 - Comparison of the basic component for griseofulvin of batches micronised with subsequent storage under respective RH with time in days.](image)

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The basic nature of the surface was studied using polar probes and Figure 5.8 displays the change in the $K_D$ values as humidity and time are varied. The values again show negative values for the samples measured at 14 days and 28 days. It is not possible to correlate the data on the basis of the $K_D$ values as previously mentioned.

RH perfusion microcalorimetry was used to determine the changes in the amorphous content of the samples held at 0, 11.3, 57.5 and 75% RH for 7, 14 and 28 days. The samples used in the study were from the same batch as that studied in the IGC to determine the surface energetic of the sample.

5.4.1.8 Determination of amorphous content for milled griseofulvin during storage

The batches were tested using the isothermal microcalorimetry as described in Section 3.1.3.3 to determine the change in amorphous content as the samples were stored in the respective RH conditions at 7, 14 and 28 days as for the IGC measurements. The same batches that were studied in the IGC measurements were analysed in the isothermal microcalorimeter unit.
5. RESULTS AND DISCUSSION

Figure 5.10 – Graph of extrapolated values using Method B from Chapter 4 to determine amorphous content for griseofulvin of stability samples post micronisation

The batch used in the experiment originated from four samples of milled active within the chambers of the Fritsch mill. The sample was split and stored within the different RH conditions and measured at the respective time points. Prior to measurement the samples were held at 0% RH for 2 hours to remove any moisture that may be adsorbed onto the surface. Samples were then flushed with nitrogen in the perfusion unit for 30 minutes at 0% RH to ensure the sample vial was free from any contaminants. A plot of the data (Figure 5.10) shows the change in the amorphous content as extrapolated from Method B from Chapter 4 for griseofulvin. Initially milled griseofulvin in the Fritsch mill for 24 hours at 300rpm gave an amorphous content of 11.11% w/w amorphous.
5. RESULTS AND DISCUSSION

<table>
<thead>
<tr>
<th>Storage time (Days)</th>
<th>0% (S.D.)</th>
<th>11.3% (S.D.)</th>
<th>57.5% (S.D.)</th>
<th>75% (S.D.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>11.11 (0.38)</td>
<td>11.11 (0.38)</td>
<td>11.11 (0.38)</td>
<td>11.11 (0.38)</td>
</tr>
<tr>
<td>7</td>
<td>4.40 (0.45)</td>
<td>4.26 (0.24)</td>
<td>0.98 (0.37)</td>
<td>0.03 (0.14)</td>
</tr>
<tr>
<td>14</td>
<td>3.50 (0.21)</td>
<td>3.74 (0.48)</td>
<td>0.94 (0.53)</td>
<td>0.44 (0.22)</td>
</tr>
<tr>
<td>28</td>
<td>3.07 (0.07)</td>
<td>3.95 (0.39)</td>
<td>0.41 (0.11)</td>
<td>0.30 (0.10)</td>
</tr>
</tbody>
</table>

Table 5.2 – Data for griseofulvin batches micronised and stored at 0%, 11.3%, 57.5% and 75% for respective time in days

The data presented using the isothermal microcalorimeter perfusion unit is tabulated in Table 5.2. The initial micronised batch is included as the sample for day 0. The first observation that can be made is the difference between the lower humidity storage conditions as compared to the higher RH conditions at 57.5% and 75%. 0% RH and 11.3% RH shows a drop in the amorphous content from 11.11% w/w to 4.40% w/w and 4.26% w/w for 0% and 11.3% RH respectively at day 7. There is a gradual decrease in the amorphous content with respect to 0% RH with a decrease observed from 4.40% w/w to 3.07% w/w from 7 to 28 days. The largest drop is noticed post micronisation during storage from day 0 to day 7. Interestingly the results for the batch held at 11.3% RH showed an initial decrease from day 7 to day 14 from 4.26% w/w to 3.74% w/w however an increase in the enthalpic data was observed from 3.74% w/w to 3.95% w/w from day 14 to day 28 respectively. This change may not be significant when taking into account the standard deviation of the sample.

The enthalpic data for the batch held at 11.3% at 28 days shows a greater response then the batch held at 0% for 28 days which shows that at 11.3% RH the surface is altered compared to conditions where the sample has no moisture. The data at 57.5% shows a decline from 11.11% w/w to 0.98% w/w from 0 to 7 days respectively. The amorphous content then remains at approximately at this level during 14 days at 0.94% w/w and then drops to 0.41% w/w after 28 days. This indicates the presence of amorphous material not crystallised or present on the surface of griseofulvin.
5. RESULTS AND DISCUSSION

5.4.2 Surface energetics of indometacin

The surface energetics of indometacin were studied to observe a change post micronisation and the subsequent change during storage as compared to the unmicronised batch. Separate batches were also micronised under the same conditions and studied over time as they were stored in different RH environments to that studied for griseofulvin.

5.4.2.1 Dispersive surface energy of milled indometacin

![Indometacin Dispersive Energy](image)

Figure 5.11 - Bar chart of the non-polar dispersive component for indometacin and the change post-micronisation during ambient storage.

Figure 5.11 displays the change in the non-polar interactions of indometacin post micronisation and subsequent storage at the respective duration. Micronisation led to an increase in the non polar interactions with an increase post milling from 33.7 mJ/m² to 46.4 mJ/m². After 1 week the values of the dispersive surface energy values show a significant decrease in the dispersive surface energy, with the largest reduction in the interaction. The active appears to be undergoing recovery and after one month and two months storage there is no significant change (ANOVA, p-value >0.05) in the values subsequent to one week. The measured response for the batch at
one and two months post-milling were still above the unmicronised batch and did not
recover to levels observed prior to micronisation in the time frame studied. The rate
of change slows after one month indicating that recovery to a lower surface energy
value is slowing.

5.4.2.2 The acid character of micronised indometacin

The polar component was investigated for indometacin and the results are highlight
the change in the acidic nature of the surface during storage post micronisation under
ambient conditions in Figure 5.12.

![Figure 5.12 - $K_a$ values for indometacin as received, indometacin micronised, micronised and
stored for 1 week, 1 month and 2 months.](image)

Post micronisation there is a large increase in the $K_A$ value for indometacin from
82.6 to 127.7 arbitrary units. In a similar observation to the griseofulvin batches the
largest drop in the acidic contributions occurs following micronisation. This is
possible due to the active undergoing recovery as amorphous material present at the
surface undergoes crystallisation. Polar groups at the surface may be exposed to a
greater extent as crystallisation proceeds which is one possible explanation. As seen
for the dispersive surface energy there is a decrease in the $K_A$ values over time,
resulting in a $K_A$ value of 111.5 which is a significant decrease from the micronised value of 127.7. The decrease in the $K_A$ values continues upon storage however not at the rate of initial storage. The change in the acidic component shows significant change (ANOVA, $p$-value <0.05) post micronisation for all batches. There is a significant increase in the $K_A$ post micronisation.

**5.4.2.3 The basic character of micronised indometacin**

- **Figure 5.13** – Graph of $K_d$ values for indometacin for batches non-micronised, micronised and stored at 1 week, 1 month and 2 months

The basic component of indometacin as received and post micronisation again shows negative values that were presented for the data for griseofulvin (Figure 5.13). Theoretically it is impossible for a material to have a negative value for the basic character, this occurs when the interaction with the polar probes is less than the interaction for the non-polar probes or a poor correlation when plotting $\Delta G_A^{SP}/AN^*$ against $DN/AN^*$. The interaction of polar probes is expected to provide higher energies of adsorption than non-polar probes as they involve both Van der Waals forces and polar interactions whereas the dispersive component does not have any acid/base contributions. The basic nature of the indometacin surface indicates the
surface has greater basic interactions post milling however it is difficult to draw comparisons as the data does not provide any meaningful results.

5.4.2.4 Stability study of micronised indometacin

5.4.2.5 IGC data for indometacin

Following the preliminary study of the surface energetics of indometacin samples were stored under varying humidity for a period of up to 28 days as previously described for griseofulvin. The data representing the non-polar interactions are presented in Figure 5.12 below.

![Graph showing dispersive surface energy comparison](image)

**Figure 5.14** – Comparison of the dispersive surface energy of indometacin batches micronised with subsequent storage under respective RH and time in days.

The values for the non-polar interaction for indometacin show a significant change whether the active is held at the lower humidity of 0% or 11.3% compared to the values seen at 57.5% and 75% (Figure 5.14). The values at the lower range of humidity decrease during storage from the value for the micronised indometacin of
46.4 mJ/m² to a significant extent (ANOVA, p-value <0.05). The effect of sorbed water on the crystallisation of indometacin in particular the difference in the crystal form at lower and higher humidities was studied in previous literature. The earlier study indicated that below 43% RH only the stable crystal form appears whereas at a higher humidity, only the metastable γ form appears (Adronis et al., 1996). This may explain the increase in the surface energy observed at higher RH storage conditions for indometacin. The active was expected to have a lower dispersive interaction due to the presence of water as previous published literature (Sunkersett, 2001) suggested the sorbed water would interfere with the probe molecules as mentioned in the measurement of griseofulvin. However, the presence of a metastable polymorph may explain the reason for the increase observed within the IGC. There is one interesting observation when observing the data that has been stored under the higher relative humidities and the data in Figure 5.9 for samples that were stored under ambient conditions. Figure 5.9 displays the dispersive surface energy for indometacin stored under ambient conditions for 28 days as 40.6 mJ/m². This is similar to the results for the lower humidity samples of 0% RH and 11.3% RH. An explanation for this could be the sample handling during the two experiments. In the stability experiments from Section 5.4.2.1 the columns were packed with glass wool on either opening and placed under ambient storage conditions. The column would not be re-packed since this would cause changes to the surface energy and disturb the packing of the column. In contrast, the samples that were tested under increasing levels of humidity samples were stored in bottles and packed at each occasion once an experiment was to be undertaken. The storage of the column in the initial experiments may not have allowed sufficient moisture into the column and may be an explanation to why the data is more closely of that to lower humidity data. This also may explain the differences in variation observed for the two studies, where the column stored under ambient conditions shows variation less than 1% however, the data for the stability study indicated greater variation >1% in the study.
5. RESULTS AND DISCUSSION

5.4.2.6 Determination of acidic character of indometacin during stability study

The acidic component present in the surface was investigated comparing the $K_a$ values for the indometacin batches as shown in Figure 5.15. There was an increase in the acidic component as the humidity increased which is again similar to the observations to that for griseofulvin. The samples stored for 14 days all showed an increased acidic component compared to the values at 28 days which indicated a decrease in the $K_a$. This increase is possibly due to the a number of factors mentioned earlier, with water playing a role in changing the charge at the surface, surface area increase post micronisation or change in the polymorphic form of the active which is likely with indometacin.
Determination of basic character of indometacin during stability study

The interaction of indometacin post micronised during storage in terms of its basic nature is shown in Figure 5.16. Compared to the non-micronised sample in Figure 5.11 (-55.2 arbitrary units) and micronised sample (-2.4 arbitrary units) there is an increase in the basic nature of the surface of indometacin during storage as a relative comparison. There is no great change when comparing the active post micronisation in terms of the humidity however when associated with an increase in the time there seems to be a general increase in the basic nature of the active. Post micronisation change in the material with a greater degree of basic sites exposed at the surface could be an explanation for this observation. There was an increase in the basic nature of indometacin for batches stored at 57.5% and 75% RH. At 11.3% RH there was an initial increase from 2.66 to 14.6 arbitrary units to 9.7 arbitrary units. It is difficult to assess whether a change in the surface energy characteristics is due to a change in a metastable polymorph as has been shown to occur at high level humidity for indometacin (Adronis et al., 1996).
5. RESULTS AND DISCUSSION

5.4.2.8 Isothermal microcalorimetry data for stability study of indometacin

Figure 5.17 - Graph of extrapolated values using Method B from Chapter 4 to determine amorphous content for indometacin of stability samples post micronisation

The samples from the stability study used in the IGC were investigated for the enthalpic response using the isothermal microcalorimeter as shown in Figure 5.16. A very interesting observation is made regarding the measured response in that samples that indicate an amorphous nature of approximately 7-10% w/w for all batches for the entire duration of the study. Previous studies have shown indometacin can form the γ polymorph form during storage and the crystallisation of indometacin after a few days can be preceded by a change in polymorphic form (Yoshioka et al., 1994). With storage temperatures close to the $T_g$ there was a presence of a second less thermodynamically stable polymorph with the stable crystal form. Indometacin starts to re-crystallise within a few days (Adronis et al., 1997) and it was not expected to show any significant enthalpic data however the results indicate a thermal event, whether is it crystallisation, or change of polymorph from the γ form to the α form or perhaps a mixture of both. There is an indication there is a thermal event occurring
for all samples, with an increase in enthalpy for the batch stored at 0% RH, 28 days after micronisation. It is possible fracture during relaxation of the material results in crack propagation as described earlier. The enthalpy result may be the thermal event of the polymorph being crystallised into the stable polymorph. Further investigations are required to analyse the polymorphic change during storage.

Table 5.3 - Data for griseofulvin batches micronised and stored at 0%, 11.3%, 57.5% and 75% for respective time in days

Table 5.3 displays the data for micronised indomethacin and the amorphous content as determined by the use of Method B in Chapter 3. Post milling the batch used had 13.43% w/w amorphous content. The amorphous content in samples decreased after the initial day however there was no significant difference observed from day 7 to day 28 at each level studied (ANOVA, p-value >0.05). For 0% RH there is a decrease to 9.73% w/w to 9.43% w/w amorphous content from day 7 to day 14. There is then an unexpected increase in the enthalpy of crystallisation at 28 days to 10.26 % w/w amorphous content. This can be explained by an increase in specific surface area of the active post micronisation. There is also a high level of enthalpy of crystallisation in the other samples which is not expected. The data shows that after 28 days all samples indicate an amorphous content of at least 7.75% w/w for the batch stored at 75% RH to 10.26% w/w for the samples stored at 0% RH. An explanation could be the presence of amorphous material beyond the outer shell of crystalline material for particles within the bulk. The surface would have characteristics of crystalline material surrounding amorphous material that may not crystallise under conditions of increasing moisture. The ethanol due to the nature of higher vaporisation may be able to penetrate further into the shell of the crystalline material at the surface and lower Tg sufficiently to cause crystallisation to occur.
CHAPTER SIX

THE INVESTIGATION INTO THE VARIATION OF GRANULE CHARACTERISTICS DUE TO THE EFFECTS OF PROCESSING AND STORAGE OF HYDROPHOBIC ACTIVES
6. RESULTS AND DISCUSSION

6.1 INTRODUCTION

Granulation is a highly important process in the manufacture of many pharmaceutical API's. Granulation has been extensively studied over the past 50 years however, there is still very little control over the process and the practice remains more of an art than a science. The process is an example of particle design where the desired attributes of the granules are controlled by a combination of formulation design (such as excipient selection, liquid properties) and process design (granulation equipment used and operating parameters). A control of the many variables involved is therefore paramount at ensuring reproducible granule characteristics. No formal methodology exists for the design of a granulation system or a system whereby a system can be developed with knowledge of the particle properties. Limitations still exist on understanding the full extent of variables that influence the final form. It is also not possible to understand how to change a formulation in order to achieve a change in the product performance during granulation. For these reasons it is highly important to understand the variability and factors occurring during granulation. PAT guidelines state that quality should be built into the system to guarantee quality built in the design of the process. Granulation is a system whereby the technique is still not fully understood. Recent advances have improved the understanding of the processes involved in granulation such as the importance of binder viscosity (Ennis et al., 1991) and powder wetting (Litster et al., 2001) however, variables not often quantified such as the amorphous content of samples during processing can be present during formulation as undetected. Earlier work by Rowe, 1989 pioneered the use of spreading coefficients and spreading behaviour by correlating the likelihood of a successful granular material if the works of adhesion and cohesion favour the formation of granule consolidation. Further work (Zajic and Buckton, 1990) has shown the ability to predict granule properties by taking into account the spreading coefficient. Once the factors that can influence granulation are understood there may be scope for controlling or predicting granule performance.
6. RESULTS AND DISCUSSION

6.2 AIMS

The purpose of the granulation study was to determine the impact of a change in the properties of griseofulvin and indometacin during micronisation and the subsequent changes to the granule characteristics. Conditioned and micronised active were micronised and granulated in a ternary granulation system with all remaining processing and formulation factors remained constant in each batch studied.

A following study was to establish the changes that occur to the formulation as the micronised actives griseofulvin and indometacin are stored at varying relative humidities for 7, 14 and 28 days and the changes that occur to the granule properties. The actives were micronised and stored under the respective RH and at each time point the material would be granulated. The changes in the granulated particle properties were studied in particular the particle size distribution of granules.

To study the change in the granule characteristics as a result of changing the bulking excipient used when formulated with conditioned and micronised griseofulvin and indometacin. Microcrystalline cellulose and α-lactose monohydrate were used as the main bulking agents and the resulting change in the particle properties were studied.

Further studies were to observe the changes in in-vitro dissolution for the granulated material and the changes in the release of active due to the micronisation of indometacin. A study was undertaken to determine how the change in material properties during storage at a range of relative humidity can impact upon the dissolution characteristics.

Another study of the granules was to determine the changes in the granule friability due to the impact of micronised material on the granulation system. The friability percentage was tested on each granule batch to understand the changes that occurred when storing the actives griseofulvin and indometacin in a range of relative humidities.
6. RESULTS AND DISCUSSION

6.3 EXPERIMENTAL METHOD

Granulation was undertaken on small scale batches in triplicate and the granule characteristics were determined to compare the differences due to the processing of the actives. The granulation procedure including the drying stage and methods to characterise the granules including in vitro dissolution testing are discussed in detail in Chapter 3.9.

6.4 RESULTS AND DISCUSSION

Initially the model granulation formulation was determined by varying the ratio of active, filler and binder and level of water content. The optimum granulation system (granules with the highest percentage of granular material between 250μm-1000μm) which was a ratio of 1:8:1 (active:filler:binder) was used to prepare 100g batches of granular material. The granulation system was used in all subsequent batches to ensure the level of each excipient remained constant throughout experiments. The binder was added to the formulation in the liquid state at a controlled rate (Section 3.11) to ensure changes in the rate of addition of binder to the dry mix would be negligible. Studies have shown the importance of the addition of the liquid binder to the formulation (Tardos et al., 1997). The study proposed that the dispersion of the binder can influence the particle size distribution. It was for this reason the rate of binder addition was controlled binder dispersion variables are negligible. The study by Tardos et al., 1997 showed that if the binder is unevenly distributed some nuclei will be more saturated than others and their growth will be preferred. The rotating mixing arm was controlled at an arbitrary speed setting of 1 which was the lowest speed setting to ensure the effect on the granules was minimal.

Initially the study of conditioned and micronised active was studied, for both indometacin and griseofulvin the material was micronised using the Fritsch mill at 300 rpm for 24 hours with 10 porcelain balls as described in Section 3.8.2. Conditioned active was prepared as according to previous work by micronising approximately 10g of active and storing the active at 100 % p/p₀ ethanol vapour.
6. RESULTS AND DISCUSSION

6.4.1 Griseofulvin granulation data

6.4.1.1 Granulation data for conditioned and milled griseofulvin / α-lactose monohydrate / PVP granules

The differences in granular particle size characteristics are shown in Figure 6.1 for granules consisting of conditioned and milled griseofulvin. The granule particle sizing was determined by automated sieving as detailed in Chapter 3.12. The percentage of granules at each sieve size was weighed and the percentage was calculated for each range. Three separate batches for each granule system were prepared and results averaged. The active griseofulvin was conditioned to ensure the most appropriate control was used in experiments by which to compare the data of the milled active against. From initial observations there is a similar distribution for both granules containing micronised and conditioned griseofulvin. Observing the data the study indicates that the granule particle size for granules consisting of
micronised griseofulvin had a higher percentage of larger aggregates particularly at the largest size range of granules $\geq 1\text{mm}$ in diameter. Statistical testing of the two sets of data indicate that there is no significant difference at the particle size range between $250\mu m$-$750\mu m$ (ANOVA, $p$-value $<0.05$) however there is a significant difference between the data at the particle size range of $x \geq 1\text{mm}$. Another important observation is the difference between the granules incorporating conditioned or milled griseofulvin at each size range in terms of the variation between the batches prepared. The batches prepared using conditioned griseofulvin indicated a lower variation at each granule size range studied. Data for the granules with micronised griseofulvin indicates a larger variation between the three batches studied and that the micronisation of the active results in an inconsistent granule particle size distribution between batches.

6.4.1.2 Granulation data for conditioned and milled griseofulvin / MCC / PVP granules

To study the changes to the formulation as the bulking agent was modified microcrystalline cellulose (Avicel PH-101) was used in place of $\alpha$-lactose monohydrate. The grade of MCC used was that used for wet granulation as it reduces the risk of overgranulation, promotes uniform granules and dries rapidly. The product specification of MCC (Avicel PH-101) indicates the loss on drying is between 3-5% and the particle size is not more than 1% w/w of particle less than 70$\mu m$ and not more than 30% w/w of particles greater than 250 $\mu m$. The particle size range for the $\alpha$-lactose monohydrate from the product specifications (Pharmatose 100M) indicated that no more than 20% of particles were less than 200 $\mu m$ and no less than 80% of particles were less than 400 $\mu m$. Compared to $\alpha$-lactose monohydrate the particle size of the bulking agent was smaller than that of the MCC used in the study. The results of the granulation of conditioned and micronised griseofulvin with MCC and PVP is shown in Figure 6.2. Earlier work by Jaiyeoba and Spring, 1980 used a ternary mixture of powders however, the particle size of the third component was found to cause changes to the particle size distribution and granule properties. The particle size of the MCC may be a crucial factor in the results observed.
Granules consisting of conditioned and micronised griseofulvin using MCC showed a skewed distribution towards smaller aggregates with approximately 80% of granules < 250μm compared to approximately 80% w/w of granules > 250μm for granules consisting of α-lactose monohydrate. This difference was largely attributed to the differences in the particle size of the bulking agent used. Micronised griseofulvin attributed to a broader peak with the largest proportion of aggregates of $0.12 > x < 0.18 \text{mm}$ which is the same mode for the granules that were prepared with conditioned griseofulvin. A significant difference is observed at the lower range of particles $x < 0.045 \text{mm}$ where there is a lower percentage, approximately 8% of granules within this size range for granules with conditioned griseofulvin compared to 17% of granules < 0.045mm for granules consisting of micronised griseofulvin. This indicates the micronisation of griseofulvin favours the formation of smaller aggregates when using MCC as the bulking agent.
6. RESULTS AND DISCUSSION

6.4.2 Griseofulvin granulation stability study data

6.4.2.1 Granule size distribution of micronised griseofulvin stored for 7 days

Studies were undertaken to determine the changes in the granulation characteristics as the properties of the active changed over time in a range of RH conditions. Griseofulvin was micronised in a Fritsch mill for 24 hours with approximately 10g stored under 0, 11.3, 57.5 or 75% RH and granulated at set time points of 7 days, 14 days and 28 days. The resulting granules were stored in glass amber bottles and the particle characteristics were tested once granulation was completed. The active granulated corresponded to the same batch of active studied in the isothermal microcalorimetry and the IGC studies in Chapter 4 and 5 respectively.

Figure 6.3 – The granule size distribution formulated with micronised griseofulvin stored at 0% (■); 11.3% (■); 57.5% (■) and 75% (■) for 7 days prior to granulation using a formulation of active, α-lactose monohydrate and polyvinylpyrillidone (PVP) in a ratio of 1:8:1 using 10% w/v water content.
6. RESULTS AND DISCUSSION

The results of the granule particle size distribution for micronised griseofulvin stored at the relative humidities of 0, 11.3, 57.5 and 75% for the set time of 7 days prior to granulation are shown in Figure 6.3. Upon observation of the data there is a clear difference in the distribution of the samples in terms of the RH conditions of storage. The particle size distribution shows a skewed distribution and the largest proportion of granules where $x > 1\text{mm}$ of approximately 30% were of the size range. As the RH is increased the pattern starts to resemble a normal bell shaped distribution with active stored at 11.3% RH and 57.5% RH having the largest proportion of granules at $0.71 < x < 1\text{mm}$ and $0.3 < x < 0.5\text{mm}$ respectively. Granules prepared with active that has been stored at 75%RH for 7 days has the largest percentage (Approximately 28% w/w) of granules in the size range of $0.25\text{μm} < x < 0.3\text{μm}$ which shows that as the RH at which the active was stored prior to granulation influenced the granulation dynamics. Micronisation and subsequent storage resulted in changes to the properties of the active and this resulted in changes to the granulation system. It is possible the variation was due to the interaction of amorphous material present at the surface of the active. From the isothermal microcalorimetry study highlighted in Figure 5.15 (Chapter 5), griseofulvin had an amorphous content of approximately 5% w/w at 7 days. A similar amorphous content (~5% w/w) was observed for the samples held at 11.3% RH for 7 days. The samples granulated at 11.3% do not show the same pattern of granule distribution as the samples held at 0% RH however do show a similar trend of larger aggregates than the samples stored at higher RH conditions. The samples held at 57.5% RH and 75% for 7 days had an amorphous content of less than 1% w/w (Figure 5.15) and the granules have a broader distribution similar to the conditioned griseofulvin that was granulated (Figure 6.1). The variability in the batches that were granulated was particularly low for the samples stored at 75%, which was a similar observation for the samples that were conditioned prior to granulation.
6. RESULTS AND DISCUSSION

6.4.2.2 Granule size distribution of micronised griseofulvin stored for 14 days

The granule particle size distributions for granules composed of griseofulvin active stored after 14 days in different RH conditions presented in Figure 6.4. On initial observations, there is a significant change in the distribution of the granules for the batch with griseofulvin micronised and stored at 0% RH at 14 days compared to the distribution at 7 days (Figure 6.3). The granules have a broader distribution with a similar pattern to the data for samples that are stored at 11.3, 57.5 and 75% RH. Statistical analysis of the groups indicate the means are not statistically different (ANOVA, p-value >0.05) and indicates the surface of the active shows no significant change with respect to the performance in granulation particle sizing data. The data for the amorphous content and the surface energetic data do not show a significant
differences from day 7 to day 14 however, the granule particle size is similar to the
trend of granules consisting of conditioned griseofulvin.

Another point to highlight from Figure 6.4 is the variation at the batch measured at
the respective RH the active was stored within. There was an increased level of
variation for griseofulvin stored at 14 days at the particle size range of \(x>1\text{mm}\)
indicating granules were less consistent in size of larger aggregates.

6.4.2.3 Granule size distribution of micronised griseofulvin stored
for 28 days

Figure 6.5 – The granule size distribution formulated with micronised griseofulvin stored at 0%  
( □ ); 11.3% ( ■ ); 57.5% ( ▼ ) and 75% ( ▶ ) for 28 days prior to granulation using a
formulation of active, \(\alpha\)-lactose monohydrate and polyvinylpyrillidone (PVP) in a ratio of 1:8:1
using 10% w/v water content. Particle size distribution of griseofulvin active stored at 0%,
11.3%, 57.5% and 75% RH for 28 days prior to granulation.
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The batches of griseofulvin were stored under varying levels of humidity for 28 days and the granule distribution data displayed in Figure 6.5. Upon observation of the data for 0% RH the granule distribution with the highest percentage were granules that were >1mm. Granulated material from the griseofulvin batch stored at 11.3% for 28 days had the highest distribution at granules between 0.3-0.5mm. 57.5% and 75% RH stored active for 28 days had granules with the highest percentage of >1mm. This indicated that all but the 11.3% granules showed granule size with the highest percentage >1mm with distributions matching the profiles for the granules with conditioned crystalline active. Variation between the granules for 0%, 11.3% and 75% decreased compared to data for that of 7 days and 14 days stored active. Granules stored at 57.5% showed the largest variation in the distribution for the batches tested.

Comparing the data to the results in the isothermal microcalorimetry study highlighted in Figure 5.8 there is a decrease in the crystallinity from approximately 1% to less than 0.5% amorphous for the sample stored at 57.5% RH for 28 days. An interesting finding is the change in the dispersive surface energy for micronised griseofulvin stored at 57.5% RH from 63.5 mJ/m² to 67.8 mJ/m² after 14 to 28 days respectively. The samples that had an increase in the dispersive surface energy showed an increase in variation at each batch such as griseofulvin stored at 75% RH for 7 days (67.8 mJ/m² dispersive surface energy). Further experimental studies must be undertaken to determine whether a difference of approximately 4 mJ/m² may cause a difference in the granulation characteristics.
6. RESULTS AND DISCUSSION

6.4.2.4 The correlation of the dispersive surface energy and the amorphous content for granules consisting of micronised griseofulvin stored at various relative humidities.

![Graph showing the relationship between dispersive surface energy and amorphous content for different RH conditions.](image)

Figure 6.6 — The relationship between the dispersive surface energy (mJ/m²) and the amorphous content of micronised griseofulvin for batches stored at varying humidities: (◊) 0%; (■) 11.3%; (▲) 57.5%; (×) 75% RH (Average of dispersive and amorphous content values taken from three separate batches) for time points of 7 days, 14 days and 28 days.

Figure 6.6 displays the relationship observed when the amorphous content (%) of the samples as investigated in Section 5.4.1.9 are correlated with the dispersive surface energy (mJ/m²) from Section 5.4.1.6. Correlating the values indicates the relationship of the two variable for griseofulvin. The values for the samples stored at lower humidities of 0 and 11.3% indicate a higher amorphous content with a lower dispersive energy and the samples stored at higher RH conditions with a lower amorphous content and higher dispersive surface energy. It was expected that samples with a higher amorphous content would have a higher surface free energy however the results indicate for the data range studied there appeared to be an inverse relationship. Studies by Krycer and Pope, 1983 and Zajic and Buckton, 1990.
have shown the possibility that differences in granulation can be correlated to the spreading coefficient. Attempts were also made to determine whether the amorphous content could be correlated to the $K_A$ of the samples and the results are presented in Figure 6.7

**6.4.2.5 The correlation of the $K_A$ to the amorphous content for granules consisting of micronised griseofulvin and stored at varying relative humidities.**

![Figure 6.7](image)

Figure 6.7 – The relationship between the amorphous content (%) of micronised griseofulvin compared to the $K_A$ for batches stored at varying humidities: (▲) 0%; (■) 11.3%; (▲) 57.5%; (▲) 75% RH (Average of amorphous content and $K_A$ values taken from three separate batches) for time points of 7 days, 14 days and 28 days.

Comparable to the observations of the correlation of the dispersive surface energy of griseofulvin to the amorphous content, the $K_A$ indicated a similar relationship. Batches stored at the higher relative humidities indicated a higher $K_A$ value which would increase the number of basic reactions occurring at the surface of the active.
Granule friability tests were undertaken to determine whether a comparison to the granule characteristics can be made to the amorphous content.

### 6.4.2.6 Griseofulvin granule friability data

<table>
<thead>
<tr>
<th>Griseofulvin</th>
<th>% fine material &lt; 250μm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Batch</td>
<td>Run 1</td>
</tr>
<tr>
<td>Conditioned griseofulvin</td>
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</tr>
<tr>
<td>Micronised griseofulvin</td>
<td>36.70</td>
</tr>
<tr>
<td>Micronised/Stored 1 Week 0% RH</td>
<td>31.30</td>
</tr>
<tr>
<td>Micronised/Stored 1 Week 11.3% RH</td>
<td>32.00</td>
</tr>
<tr>
<td>Micronised/Stored 1 Week 57.5% RH</td>
<td>27.80</td>
</tr>
<tr>
<td>Micronised/Stored 1 Week 75% RH</td>
<td>25.20</td>
</tr>
<tr>
<td>Micronised/Stored 2 Week 0% RH</td>
<td>30.20</td>
</tr>
<tr>
<td>Micronised/Stored 2 Week 11.3% RH</td>
<td>32.70</td>
</tr>
<tr>
<td>Micronised/Stored 2 Week 57.5% RH</td>
<td>23.60</td>
</tr>
<tr>
<td>Micronised/Stored 2 Week 75% RH</td>
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</tr>
<tr>
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<td>31.80</td>
</tr>
<tr>
<td>Micronised/Stored 4 Week 11.3% RH</td>
<td>32.00</td>
</tr>
<tr>
<td>Micronised/Stored 4 Week 57.5% RH</td>
<td>24.20</td>
</tr>
<tr>
<td>Micronised/Stored 4 Week 75% RH</td>
<td>24.70</td>
</tr>
</tbody>
</table>

Table 6.1 – Table displaying the percentage of fines <250μm for griseofulvin milled and stored pre granulation under specified conditions.

The results from the friability study are shown in Table 6.1 where the percentage of granules <250 μm after rotation are shown for triplicate batches. The average was taken and plotted to highlight the differences graphically and shown in Figure 6.6. On initial inspection of the micronised batch compared to the conditioned batch there is a greater percentage of fines for the batches using micronised griseofulvin as
opposed to conditioned griseofulvin, with a percentage friability of 21.63 (0.76) and 35.53 (1.20) for the batches using conditioned and micronised griseofulvin respectively.

Figure 6.8 - Friability of granules (percentage of fines <250μm after friability testing) containing micronised griseofulvin and stored under 0, 11.3, 57.5 or 75% for 7, 14 or 28 days.

Figure 6.8 displays the differences in the percentage of granular material < 250μm after rotating for 10 minutes in the friability tester at 25rpm for 10 minutes. Initially conditioned and micronised active were granulated with lactose and PVP binder in the aqueous state. The micronisation of griseofulvin results in a 14% increase in friability. The increase in friability may be related to the increase in the granule size observed during the granule sizing stability studies. The increase in the friability may be attributed to the increase in size however, they are held together by weaker bonds after the active has been micronised.

This indicates that micronisation of the active results in larger aggregates held together by weaker bonds. Upon observation of the granulation data post storage under various humidities there is an increase in the friability for samples for samples...
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held at the lower humidities when compared to the lower friability observed for actives that were stored at the higher humidities for the set length of time. The friability characteristics for the samples held at 57.5% and 75% RH prior to granulation show a level of friability similar to the active that was conditioned prior to granulation.

6.4.2.7 The correlation of the friability and the amorphous content for granules consisting of griseofulvin micronised and stored at varying relative humidities

![Graph showing the relationship between amorphous content and friability for different humidities.]

Figure 6.9 – The relationship between the amorphous content (%) of micronised griseofulvin compared to the friability ( % of granules passing through 250μm sieve) for batches stored at varying humidities: (♦) 0%; (■) 11.3%; (▲) 57.5%; (▲) 75% RH (Average of dispersive and amorphous content values taken from three separate batches) for time points of 7 days, 14 days and 28 days.
Figure 6.9 highlights the relationship between the amorphous content of the samples of micronised griseofulvin at the respective storage conditions and the friability of the granules by taking into account the percentage of material passing through a 250 μm sieve after rotation in a friability tester. The results indicate approximately 5% increase in friability of samples with a higher amorphous content. The samples with an increased amorphous content were the samples held at the lower RH environments specifically the samples stored at 0% RH and 11.3% RH. Samples held at the higher relative humidities and with less than 1% amorphous content had a friability of approximately 25% w/w. This indicated the re-crystallisation of the material during storage resulted in a change in the friability of the final form.
6. RESULTS AND DISCUSSION

6.4.3 Indometacin granule data

6.4.3.1 Granulation data for conditioned and milled indometacin / \(\alpha\)-lactose monohydrate / PVP granules

Indometacin was granulated using lactose, active and PVP in a 8:1:1 ratio as 100g batches as mentioned for griseofulvin. Micronised indometacin and conditioned indometacin were granulated in triplicate batches and the average of the particle size distributions shown in Figure 6.10.

![Figure 6.10 - The granule size distribution formulated with conditioned indometacin ( ▲ ) and micronised indometacin ( ▼ ) using a formulation of active, \(\alpha\)-lactose monohydrate and polyvinylpyrillidone (PVP) in a ratio of 1:8:1 using 10% w/v water content.](image)

Conditioned indometacin was used as the control to compare the micronised indometacin batches to negate any differences that particle size may have on the granulation process. Initial observations show that conditioned active once granulated, shows a skewed distribution with a mode of 0.7\(<x<1\) mm. Micronised active that was granulated showed a similar distribution to the conditioned
indometacin, being more skewed towards larger particles with a mode of $x > 1$mm particle size. There was a statistical difference between the batches that were micronised and granulated compared to the conditioned active granulated for the granule sizes greater than 1mm in diameter (ANOVA, $p$-value <0.05). For the other size ranges there was no statistical difference observed (ANOVA, $p$-value >0.05). The standard deviation at each granular size level also shows a difference between conditioned and micronised active once granulated. At each granule size level there is less deviation from the average for the conditioned active that was granulated then the micronised batches. This indicated that batch variation is greater when using micronised indometacin as opposed to conditioned indometacin.

Initially the batches that were tested used indometacin conditioned via exposure to ethanol 100% $p/p_0$ vapour and milled indometacin active. The data which is graphically represented in Figure 6.10 compares the differences in the particle size distribution for the two batches. The particle size distribution for conditioned indometacin which was granulated shows a broader distribution then the milled indometacin. This indicates the granules containing the conditioned indometacin has greater spread of particle sizes then the granules containing milled indometacin which favours larger agglomerates. There is an increase in the proportion of granules in the size range of $x > 1$mm from approximately 12% to 26% when using micronised indometacin as compared to the results of the granules containing conditioned active.

The variation between the different batches using the same active also shows a significant finding. The granules containing conditioned indometacin has a lower variation in the particle distribution then that observed for the granules containing the milled indometacin. Granules with milled indometacin show an increase in the variation in the three batches tested compared to the three batches containing conditioned active. This can be explained as the surface of the active post milling has greater variation in terms of its reactivity during the particle agglomeration phase. The higher energy state of the amorphous regions propagated during milling shows a difference in particle characteristics during granulation. Granulation of the active using MCC as the bulking agent was undertaken as previously undertaken for griseofulvin.
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6.4.3.2 Granulation data for conditioned and milled indometacin / MCC / PVP granules

The particle size distribution of granules that contained micronised and conditioned indometacin using MCC and PVP is displayed in Figure 6.11. The use of MCC as the filler shows the granule particle size has a mode of particles between 0.045 and 0.075 for conditioned granules and 0.12 and 0.075 for micronised indometacin granules. In contrast to the granules using lactose as the filler the granule particle size was skewed towards granules with a lower level of granule consolidation. Over 80% of the granule size for all batches containing micronised or conditioned active were granules with a size <250μm. This indicated poorly developed granules and can be attributed to the interactions of MCC, PVP and active. As observed in the batches using lactose as a bulking agent, there was a greater variation in particle size at each range when the micronised active was granulated as compared to the batches using conditioned active. This shows an increase in the variation as the active is milled.
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highlighting the changes in the granulation characteristics due to a change in the surface properties of the material.

6.4.4 Indometacin Stability Study

Indometacin samples were micronised using the Fritsch high energy ball mill for 24 hours and the batches were stored under 0, 11.3, 57.5 and 75% RH prior to granulation at specified time points of 7, 14 and 28 days. Granulation size distribution data and friability studies were undertaken to determine the change in the granule characteristics due to storage conditions.
6. RESULTS AND DISCUSSION

6.4.4.1 Granule size distribution of micronised indometacin stored for 7 days

Figure 6.12 - The granule size distribution formulated with micronised indometacin stored at 0% (■); 11.3% (■); 57.5% (■) and 75% (■) for 7 days prior to granulation using a formulation of active, α-lactose monohydrate and polyvinylpyrillidone (PVP) in a ratio of 1:8:1 using 10% w/v water content.

Figure 6.12 shows the granule particle size distribution for indometacin which was micronised and then stored under 0, 11.3, 57.5 and 75% RH prior to granulation for 7 days. Comparing the data set there is no significant difference (ANOVA, p-value >0.05) between the four humidity levels. One observation that is noted is the increase in the variability in the batches of micronised indometacin stored at 0% and 11.3% storage conditions. This indicates at the lower humidity levels there is an increase variability and less consistent granular particle size distribution when active is stored at low level humidity for micronised indometacin. The distributions of the granules containing indometacin stored at the various humidity conditions had a similar distribution to the granule distribution of conditioned indometacin. A broader
distribution with a lower percentage of larger aggregates, particularly above the 1 mm size range.

6.4.4.2 **Granule size distribution of micronised indometacin stored for 14 days**

![Graph showing granule size distribution](image)

Figure 6.13 - The granule size distribution formulated with micronised indometacin stored at 0% ( ), 11.3% ( ), 57.5% ( ) and 75% ( ) for 14 days prior to granulation using a formulation of active, α-lactose monohydrate and polyvinylpyrillidone (PVP) in a ratio of 1:8:1 using 10% w/v water content.

Figure 6.13 displays the particle size distribution for micronised indometacin stored at the relative humidities of 0, 11.3, 57.5 and 75% RH for 14 days prior to granulation. Statistical analysis of the data groups indicated no statistical differences (ANOVA, p-value > 0.05) for each particle size range. This indicated the storage of the active in the varying ranges of humidity had no impact upon the granule size distribution. There was also no significant differences in the variation at each data set and the distribution matched closely to the distribution observed when granulating...
conditioned indometacin. Similar to the results observed for indometacin stored at 7 days there was little difference to the distributions. The largest proportion of the granules are sized between $0.3 < x < 1\text{mm}$ which in total accounts for approximately 75% of the granule percentage.

6.4.4.3 **Granule size distribution of micronised indometacin stored for 28 days**

- Milled indometacin - stored 0%RH 28 days - granules (lactose/PVP)
- Milled indometacin - stored 11.3%RH 14 days - granules (lactose/PVP)
- Milled indometacin - stored 57.5%RH 14 days - granules (lactose/PVP)
- Milled indometacin - stored 75%RH 14 days - granules (lactose/PVP)

Figure 6.14 - The granule size distribution formulated with micronised indometacin stored at 0% ( ), 11.3% ( ), 57.5% ( ) and 75% ( ) for 28 days prior to granulation using a formulation of active, $\alpha$-lactose monohydrate and polyvinylpyrillidone (PVP) in a ratio of 1:8:1 using 10% w/v water content.

Figure 6.14 displays the granule size distribution containing micronised indometacin that was stored under the various humidities and granulated after 28 days. The distribution of granules exhibited a similar pattern to that observed for indometacin stored at the elevated humidities for 7 days and 14 days. There was no statistical difference (ANOVA, p-value >0.05) between the ranges of humidities under which...
the active was stored under prior to granulation. A greater insight into the differences in the active properties were gained by correlating the amorphous content and the surface energetic data for indometacin.

6.4.4.4 The correlation of the dispersive surface energy and the amorphous content for granules consisting of micronised indometacin stored at various relative humidities

![Figure 6.15](image)

Figure 6.15 – The relationship between the amorphous content (%) of micronised indometacin compared to the dispersive surface energy (mJ/m²) for batches stored at varying humidities: (○) 0%; (■) 11.3%; (▲) 57.5%; (×) 75% RH (Average of amorphous content and dispersive surface energy values taken from three separate batches) for time points of 7 days, 14 days and 28 days.

The data correlating the amorphous content (%) of micronised indometacin which was stored under the RH conditions of 0, 11.3, 57.5 and 75% to the dispersive surface energetic data are shown in Figure 6.15. The amorphous content (%) was calculated as outline in Section 5.4.2.9 and the data shows the measured amorphous content of a triplicate batch of sample at each data point. For all the batches studied the amorphous content fell within the range of 7.5 to 10.5% amorphous content w/w.
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There was no significant change to the dispersive surface energy of the samples in terms of the days storage however there is a gradual decrease for the samples in terms of amorphous content % for the batches held at 0% RH and 11.3% RH. There is very little change to the amorphous content observed for indomethacin stored at 75% for the duration of 7 to 28 days. It has been shown in previous studies that amorphous indomethacin at 84% RH and 30°C takes around 80 days for complete crystallisation and the time scale is longer than the time scale studied experimentally (Adronis et al., 1997). The experimental results indicate the amorphous form is present on the surface and more interestingly higher RH conditions seems to indicate a higher dispersive surface energy compared to lower RH storage conditions. The changes in the dispersive surface energy did not result in observable differences to the granule distribution data. Further studies to correlate the data of $K_A$ to the amorphous content was undertaken and results can be shown in Figure 6.16.

6.4.4.5 The correlation of the $K_A$ to the amorphous content for granules consisting of micronised indomethacin and stored at varying relative humidities

![Graph showing the relationship between the amorphous content (%) and $K_A$ for batches stored at varying humidities: (♦) 0%; (□) 11.3%; (▲) 57.5%; (✗) 75% RH (Average of 182)]

Figure 6.16 – The relationship between the amorphous content (% calculated using Method A of analysis from Section 3.1.3.3) of micronised indomethacin compared to the $K_A$ for batches stored at varying humidities: (♦) 0%; (□) 11.3%; (▲) 57.5%; (✗) 75% RH (Average of 182)
amorphous content and $K_a$ values taken from three separate batches) for time points of 7 days, 14 days and 28 days.

The correlation between the $K_a$ and the amorphous content (%) indicates a general negative linear relationship between the ranges studied. As the amorphous content is decreased there is an increase in the $K_a$ for indomethacin. Observing the samples that were stored at 11.3% and 57.5% RH there appeared to have very similar amorphous content % for the batches studied. This result shows how the increase in the amorphous content decreases the acidic contributions at the surface. The surface as it becomes more amorphous (and subsequently less acidic) through processing will favour acidic interactions more strongly at the surface than material which is crystalline.

### 6.4.4.6 Indomethacin granule friability data

<table>
<thead>
<tr>
<th>Batch</th>
<th>% fine material &lt; 250μm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Run 1</td>
</tr>
<tr>
<td>Conditioned indomethacin</td>
<td>27.80</td>
</tr>
<tr>
<td>Micronised indomethacin</td>
<td>34.30</td>
</tr>
<tr>
<td>Micronised/Stored 1 Week 0% RH</td>
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</tr>
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</tr>
<tr>
<td>Micronised/Stored 4 Weeks 75% RH</td>
<td>32.00</td>
</tr>
</tbody>
</table>

Table 6.2 – Table displaying the percentage of fines < 250μm for indomethacin milled and stored pre granulation under specified conditions.
Granule friability was tested as described in Section 3.15 for indomethacin and the results are presented in Table 6.2. On initial observation there is an increase in the friability between conditioned and micronised indomethacin from an average of 24.87 (2.90) to 31.77 (2.60) for the conditioned indomethacin and micronised indomethacin respectively. The micronisation process increases the percentage of friable material by approximately 6% w/w and this is a significant proportion of granules <250μm. This indicates the presence of the micronised material influences the level of friability in indomethacin granules.

![Graph showing friability of granules](image)

**Figure 6.17 – Friability of granules (percentage of fines < 250μm after friability testing)** containing micronised indomethacin and stored under 0, 11.3, 57.5 or 75% for 7, 14 or 28 days.

The data is also presented in a graphical form in Figure 6.17 where the differences between the conditioned indomethacin and micronised indomethacin are highlighted in terms of granule friability. The samples that were tested where the active was stored under the various humidities did not exhibit any statistical significant differences in terms of friability (ANOVA, $p$-value >0.05). However, there was a clear gradual decrease in the friability for samples at 28 days where there is a gradual decrease in
the friability % as the RH is increased. The values of the indomethacin stored under 0% RH for 28 days and the micronised indomethacin stored at 75% after 28 days showed a statistically significant change in the friability of granules with a change from approximately 35% friability to 30% friability respectively.

6.4.4.7 The correlation of the friability and the amorphous content for granules consisting of indomethacin micronised and stored at varying relative humidities

![Graph showing the relationship between amorphous content and friability](image)

Figure 6.18 – The relationship between the amorphous content (%) of micronised indomethacin compared to the friability (%) for batches stored at varying humidities: (◊) 0%; (■) 11.3%; (▲) 57.5%; (X) 75% RH (Average of amorphous content and friability (%) values taken from three separate batches) for time points of 7 days, 14 days and 28 days.

Figure 6.18 shows the correlation between the amorphous content (%) and the friability of granules consisting of indomethacin which has been stored under the respective RH and time points prior to granulation. From the data there was no significant difference between the range of amorphous levels observed which fell between 7.5 to 10.5%. This 3% range may not be large enough for a change to be evident, however there is no statistical difference (ANOVA, p-value >0.05) between the data. Friability accounted for between 30 to 40% for all the data sets studied which indicated a large proportion of granules were held together by weak bonds.
6. RESULTS AND DISCUSSION

6.4.5 Dissolution

6.4.5.1 Dissolution of granules consisting of griseofulvin

To maintain sink conditions for griseofulvin not more than 3mg could be used in the formulation. There was no possibility of altering the formulation. Using the minimum required weight of the granules required 30mg which when used for the dissolution results would not be representative of the population. I tried to use 30mg but it resulted in large variations in the dissolution profile as only a small amount of granular material could be used which caused large errors during sample handling.

6.4.5.2 Dissolution of granules consisting of conditioned indometacin and micronised indometacin

![Graph showing dissolution profile of conditioned and micronised indometacin](image)

Figure 6.19 – Dissolution of conditioned active (■) and micronised active ( ■) indometacin release from granules of active, α-lactose monohydrate and PVP as an aqueous binder with 10% w/v water content.
Figure 6.19 displays the release of indometacin from granules prepared using active which has been conditioned (to give a crystalline material) compared to granules that have been prepared using milled indometacin. The rate of release of indometacin granules with micronised indometacin indicates a faster rate of release than granules prepared from conditioned active. Either the rate of release is faster because of the active being different in size or due to the difference in the amorphous content which may be present in granules. The batches were not tested in capsules as the capsules retarded the dissolution process as the active adhered to the capsule surface and limited dissolution into the medium. Tabletting was not undertaken as this would alter the surface energetics during tabletting. Tabletting would include a number of changes that may occur to the surface properties of the material that may cause further complications to the energetic state of the formulation.

6.4.5.3 Dissolution of granules consisting of micronised indometacin stored at various humidities for 7 days prior to granulation.

![Graph showing dissolution of stored indometacin under various RH after 1 week storage](image)

Figure 6.20 – Dissolution of stored indometacin under various RH after 1 week storage

Granules that were prepared in the stability study as previously mentioned in Section 6.4.4 were tested for in-vitro dissolution to determine whether there a difference in
the release of drug due to the micronisation and subsequent storage of indometacin. There was no clear distinction between the batches and no significant difference (ANOVA p-value >0.05) between the data sets. The rate of release from all four formulations were indistinguishable in terms of the rate of release and dissolution profile. The formulations that contained indometacin that was stored at 11.3% RH and 57.5% RH for one week gave a higher than expected total active concentration of approximately 110%. The batches that were stored at 0% and 75% RH gave approximately the expected drug release of 100% after 30 minutes. A reason any differences might not be observable is the rapid rate of release from granules within the dissolution chamber. Unfortunately attempts to retard the release such as the use of capsules did not give reproducible data.

6.4.5.4 Dissolution of granules consisting of micronised indometacin stored at various humidities for 14 days prior to granulation

Figure 6.21 – Dissolution of stored indometacin under various RH after 2 weeks storage

Figure 6.21 presents the dissolution data for the granules containing indometacin that was stored at 0, 11.3, 57.5 and 75% RH for 2 weeks. The results as shown for the 1 week data show there is no statistical difference between the data sets (ANOVA, p-
value >0.05). Indometacin showed no change in the dissolution as samples were stored under various humidities prior to granulation.

6.4.5.5 Dissolution of granules consisting of micronised indometacin stored at various humidities for 28 days prior to granulation

![Graph showing dissolution of indometacin under various RHs after 4 weeks storage](image)

**Figure 6.21 – Dissolution of indometacin under various RHs after 4 weeks storage**

Figure 6.2.1 shows the graph for indometacin stored under various RH environments prior to granulation however there was again no significant difference in the data for dissolution. It is possible there is very little difference in the dissolution profile of indometacin when stored at various RH environments. There is a change that is observed during micronisation and during the surface energetic data as highlighted in Chapter 5 however this was not reflected on the granulation data or the dissolution data.
7. CONCLUSIONS

Amorphous Quantification Conclusions

The investigation has shown it is possible to use the organic vapour ethanol within the perfusion microcalorimeter to study the crystallisation of three hydrophobic actives. A quantification technique was developed using the perfusion microcalorimeter to determine amorphous content from 0.5-5% w/w amorphous. The work highlighted the significance of the measurement, in particular understanding the processes occurring during crystallisation and the data extrapolation used to extract the relevant data for the enthalpy of crystallisation. Two methods to calculate this data were used with Method B giving very accurate and reproducible measurements by taking into account the heat change of desorption and using this data to eliminate effects such as sample wetting, sample adsorption/absorption and wetting of the internal measuring ampoule. Significant observations were made using the data, particularly the assumption budesonide was partially amorphous as received. The data from both methods of analysis were used to elucidate an event occurring during the sorption phase of the experiment.

The quantification experiments for the actives enabled the used of the calibration curve to determine the amorphous content of three types of milling techniques. Griseofulvin and indometacin were milled using an air jet mill, vibration ball mill and a rotary mill. Factors such as mill time and milling pressure were varied for each technique and analysis of the data showed an increase in amorphous content as the intensity of milling parameters were increased. In particular the rotary ball mill indicated an amorphous content of 10.49% w/w for griseofulvin and 17.28% w/w for indometacin respectively. The amorphous content present in the samples fell outside the range of that studied to construct the calibration curve, however it was assumed the enthalpy of crystallisation and amorphous content would be linear. The quantification indicates a large proportion of the active exists in the amorphous state post micronisation that may have significant implications in processing.

If further studies could be undertaken I would investigate the use of the isothermal microcalorimeter in a ternary system whereby a mixture of actives and/or excipients could be
investigated using the RH perfusion unit and forming a method that could be used to extract amorphous content information on ternary mixtures.

Griseofulvin Surface Energetic Conclusions

The IGC studies of griseofulvin showed that the surface properties of the active can largely vary with the milling technique used. There were large differences between different milling techniques employed. Storage at varying humidities resulted in large differences in the surface energies of batches of micronised griseofulvin. The processing of griseofulvin resulted in an increase in the acidic component of the surface. Upon storage at a range of RH conditions the dispersive surface energy was found to increase as the humidity increased. The amorphous content of griseofulvin was also measured using the perfusion isothermal microcalorimeter. Samples held at lower humidity were found to have a low amorphous content (~3-4% w/w amorphous) after 28 days storage. Samples held at higher humidity indicated the batches had an amorphous content of < 1% w/w amorphous after 28 days storage. IGC is useful at identifying changes at the surface however it is important to use other techniques to fully understand the variation in surface properties of an active during micronisation and storage.

Indometacin Surface Energetic Conclusions

The surface energetic of indometacin showed some very interesting results. Upon milling there was an increase in the non-polar interactions at the surface of the active. Subsequently storage at ambient conditions resulted in a decrease of the dispersive surface energy. The surface post milling also indicated a more acidic surface with the basic nature also increasing during storage at ambient conditions post storage. The dispersive surface energy for samples stored at lower humidity decreased however for higher humidity conditions the dispersive surface energy increased. As the humidity increased there was an increase in the acidic and basic character of the batches. Isothermal microcalorimetry indicated the batches were still amorphous and all samples indicated an amorphous content of between 7-10% w/w. The IGC data indicated a difference in the dispersive surface energy, possibly due to a change in the polymorph of indometacin at higher relative humidities. The investigation has showed how a change in processing can alter the surface properties of an active and how changes during storage can result in vastly different properties.
7. CONCLUSIONS

Granulation Conclusions

The granulation study gave an insight into the changes that occur at a particulate level as variation in the properties of an active can lead to changes in the final formulation. The study highlighted how a change in the material properties can impart a significant difference to the granule characteristics. Conditioned griseofulvin and conditioned indometacin when granulated had a lower percentage of larger aggregates than the micronised active. The friability of granules (% granules <250 μm) also decreased when using conditioned active in the formulation. Micronised samples formed larger aggregates and had a higher percentage of friability for both of the actives indometacin and griseofulvin.

Indometacin and griseofulvin were micronised and then stored under varying levels of RH prior to being granulated at set time points of 7 days, 14 days and 28 days. When studying the granules consisting of griseofulvin, active that was stored at the lower humidities performed similar to that of micronised material, as the majority of granules consisted of larger aggregates >1mm. The same relationship was not observed for indometacin as there was no significant difference in the granules as active was stored at increasing levels of RH.

An observation in the changes to the granular characteristics as the bulking agent was changed also highlighted the importance of excipient selection. Microcrystalline cellulose was granulated with active and PVP and showed to consist of over 80% w/w granules <250μm. The difference in particle size of MCC largely attributed to the differences observed however, granules consisting of α-lactose monohydrate had less variation between batches indicating a more favoured system of granulation for the two hydrophobic actives.

The in-vitro dissolution test for indometacin indicated there was an increase in the rate of dissolution for granules consisting of micronised indometacin. This increase in the rate of dissolution may be attributable to the increased amorphous content post milling compared to the conditioned form when granulated. No statistical differences were observed for samples stored under increasing levels of RH prior to granulation.
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