Characterisation of the amorphous solid state using solvent vapour induced transitions

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

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2005
This thesis describes research conducted in the School of Pharmacy, University of London January 2002 and December 2005 under the supervision of Prof. 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 texts herein and have clearly indicated by suitable citation any parts of this dissertation that has already appeared in publication.

Signature:  
Date:
Abstract

There is increasing awareness about the amorphous form or disordered structure of material. The amorphous state is studied widely, due to two reasons; first, it is encountered without choice during many processes (e.g. milling/micronization) and second due to its advantages over the crystalline form (e.g. solubility). The important aspect of the amorphous form is its metastability (tendency to transform into a thermodynamically stable crystalline form). The current studies on the amorphous solid state involve, i) quantifying the disorders in otherwise ordered crystalline structures; ii) identifying the conditions under which its transformation into an ordered crystalline form could be controlled and iii) stabilizing the amorphous form by formulating as a solid dispersion. The characterisation of the amorphous state is mainly based on thermal analytical techniques (DSC) to estimate glass transition temperature. Other techniques, which are used routinely to study the amorphous state, involve spectroscopy (XRPD, FT-Raman, FT-IR, and Terahertz), microcalorimetric analysis (Solution calorimetry) and vapour sorption analysis (DVS).

The aim of the thesis was to identify the techniques to characterise the amorphous state under the controlled conditions of temperature and humidity. These techniques would then be used to identify the conditions for the zero molecular mobility in the amorphous model substances selected for the study.

The heating rate dependence of the glass transition was studied for amorphous indometacin. These studies were then used to calculate the fragility or the strength parameter and the activation energy (of relaxation process) for the amorphous solid. These parameters can be used to compare the relative stability of the different glasses. The StepScan DSC could successfully distinguish the process of aging in the amorphous states of indometacin, nifedipine and lactose. It was also observed that below a certain temperature for each amorphous state the relaxation time became extremely high, this temperature could be correlated to the Kauzmann temperature (Tk).

The various transitions induced in the amorphous state of indometacin and lactose due to RH ramp were studied using DVS, perfusion calorimetry and IGC. The correlation of transitions from DVS was based on changes in weight gain profile as compared to changes in power output signal from perfusion calorimetry and changes in retention volume and pressure drop by using IGC. For amorphous lactose a sequence of transitions viz. mobility onset, glass transition, collapse and crystallisation could be followed as the RH was ramped. It was possible to characterise each transition with respect to a critical RH (%cRH) required for its induction e.g. %cRHg and %cRHcry as %RH required to induce glass transition and crystallisation respectively. The values of %cRH obtained using different techniques matched with each other.

In the case of amorphous indometacin, the preferential surface plasticisation effect of sorbed water was demonstrated using IGC. Although the glass transition could be observed on the surface of amorphous particle, no spontaneous crystallisation could be seen with the RH ramp. Using a serial ramp of alcohol pressure (e.g. methanol, ethanol and propanol) by perfusion calorimetry it was possible to demonstrate glass transition and crystallisation in amorphous indometacin; these transitions could be observed from the power output signal obtained.

The different values of critical solvent pressures obtained at different temperatures were used to estimate Tk for amorphous indometacin and lactose.

NIR studies demonstrated changes in amorphous nifedipine for the storage at room temperature, which otherwise would have been missed while characterising by IGC, DVS and TAM. The various techniques used in this work could be used very effectively to study the amorphous state.
Dedicated to my beloved family
Acknowledgement

Firstly I'd like to acknowledge my supervisor, Prof Graham Buckton, for his constant support and guidance. I would also like to thank Nigel, Brian, Kim and Ian from WWPP, GSK, for the scientific support and help during my industrial placement.

Thanks must go to GSK for funding this work and School of Pharmacy for making an avenue toward this PhD.

Special thanks to Keith, for all his technical support during my work in the labs.

I would like to dedicate this thesis to my grandma, my mum and my dad. I am thankful to my family, my mum and dad, my brother, uncle and aunts for constant support and the faith they expressed in me.

I would like to thank my colleagues at work Ketan, Laurent, Abi, Dima, Richard, Ann, Amina, Emma, John and colleagues at my home Orawan, Pla, Kuldeep for making my time enjoyable. Special thanks to Orawan for being a great friend.

This acknowledgement cannot be complete without mentioning Owen Shephard. The demonstrations on Sterile products was a great fun and good break from the PhD work.

Lastly thanks must go to my friends, Dnyanesh, Sanjive and Bipin you all have been a great source of motivation for me.
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<th>Description</th>
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</thead>
<tbody>
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<td>VTF</td>
<td>Vogel-Tamman-Fulcher equation</td>
</tr>
<tr>
<td>KWW</td>
<td>Kohlrausch-Williams-Watts equation</td>
</tr>
<tr>
<td>AGV</td>
<td>Adam-Gibbs-Vogel (AGV) equation</td>
</tr>
<tr>
<td>BET</td>
<td>Brunauer-Emmett-Teller equation</td>
</tr>
<tr>
<td>BCS</td>
<td>Biopharmaceutics classification system</td>
</tr>
<tr>
<td>DSC</td>
<td>Differential scanning calorimetry</td>
</tr>
<tr>
<td>XRPD</td>
<td>X-ray powder diffraction</td>
</tr>
<tr>
<td>MDSC</td>
<td>Modulated temperature differential scanning calorimetry</td>
</tr>
<tr>
<td>DVS</td>
<td>Dynamic vapour sorption</td>
</tr>
<tr>
<td>NIR</td>
<td>Near infrared spectroscopy</td>
</tr>
<tr>
<td>IGC</td>
<td>Inverse gas chromatography</td>
</tr>
<tr>
<td>DMA</td>
<td>Dynamic mechanical analysis</td>
</tr>
<tr>
<td>TSC</td>
<td>Thermally stimulated current spectroscopy</td>
</tr>
<tr>
<td>NMR</td>
<td>Nuclear magnetic resonance</td>
</tr>
<tr>
<td>DEA</td>
<td>Dielectric analysis</td>
</tr>
<tr>
<td>MFCs</td>
<td>Mass flow controllers</td>
</tr>
<tr>
<td>TCD</td>
<td>Thermal conductivity detector</td>
</tr>
<tr>
<td>FID</td>
<td>Flame ionization detector</td>
</tr>
<tr>
<td>MITAT</td>
<td>Moisture induced thermal activity trace</td>
</tr>
<tr>
<td>DTIC</td>
<td>Double twin isothermal calorimeter</td>
</tr>
<tr>
<td>%RH</td>
<td>Relative pressure of water</td>
</tr>
<tr>
<td>%cRHg</td>
<td>Critical relative pressure of water required to induce glass transition</td>
</tr>
<tr>
<td>%cRHcry</td>
<td>Critical relative pressure of water required to induce crystallisation</td>
</tr>
<tr>
<td>%RM</td>
<td>Relative pressure of methanol</td>
</tr>
<tr>
<td>%cRMg</td>
<td>Relative pressure of methanol to required induce glass transition</td>
</tr>
<tr>
<td>%cRMcry</td>
<td>Relative pressure of methanol to required induce crystallisation</td>
</tr>
<tr>
<td>%RE</td>
<td>Relative pressure of ethanol</td>
</tr>
<tr>
<td>%RP</td>
<td>Relative pressure of propanol</td>
</tr>
<tr>
<td>SNV</td>
<td>Standard normal variate</td>
</tr>
<tr>
<td>DMF</td>
<td>Dimethyl formamide</td>
</tr>
</tbody>
</table>
Chapter 1  
Introduction
1.0 Introduction

Solid dosage forms are the most frequently used form of drug delivery. Dosage forms in which the drug is in solid state include tablets, capsules, dry powder inhalers, dispersions or suspensions.

The active ingredient in the solid dosage can have various forms (e.g. polymorphs or amorphous). It is important from the formulation and the regulatory point of view that the dosage form consists of the most pure and stable form of the active. Many times the formulator is tempted to use the meta-stable form (e.g. amorphous state or meta-stable polymorph) because of the wide range of advantages over the stable form. In such cases, it is important to understand the vulnerability of the meta-stable form to various pharmaceutical processes. It is also important that the selected form stays stable over the shelf life of the product.

Whereas to select the most suitable form of the active for the dosage form under development is important from the innovators point of view, to find and stabilize the metastable forms may be an advantage from the generic point of view. There is always an ongoing race to better understand the solid state either by developing new techniques to characterise the existing solid state or to use the existing techniques to discover new forms of the active.

The use of the amorphous form of the active is mainly impeded by the meta-stability and its tendency to transform into the more stable crystalline form. In this thesis an attempt is made to better understand the amorphous state from the pharmaceutical point of view.
1.1 Solid state of matter

Solid state of a matter is characterised by interlocking of the constituent molecules with respect to each other. The constituent molecules are tightly held together by means of intermolecular linkages (mainly strong hydrogen bonds, and Van der waals forces of attraction) (Martin, 1993a). Molecules in liquid do exhibit intermolecular bonds but they are comparatively weaker than in the solids. Liquids exist in a sort of dynamic state where old bonds are continuously broken and replaced by new bonds. In other words molecules exhibit significant translational and vibrational entropy. This is not the case with solids. Molecules in the solid state can exhibit only vibrational entropy and translational entropy to a very limited extent.

1.1.1 Polymorphs, solvates and amorphous forms

The solid state, depending on how the molecules are arranged could be further classified (Byrn et al., 1999) as follows.

<table>
<thead>
<tr>
<th>Solid State</th>
</tr>
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<tbody>
<tr>
<td>Ordered structure (Crystalline)</td>
</tr>
<tr>
<td>Disordered Structure (Amorphous)</td>
</tr>
<tr>
<td>Polymorphs</td>
</tr>
<tr>
<td>Solvates</td>
</tr>
</tbody>
</table>

**Crystal:** Homogeneous portion of matter that has a definite, orderly atomic (hence molecular) structure. It is also an outward form bounded by smooth plane surfaces which are arranged symmetrically.

**Amorphous** (Glassy material): Materials that are solids whose atoms or molecules do not adopt a crystalline lattice, but which nevertheless cannot easily move past one another.

**Polymorphs:** When two crystals have a same chemical composition but different internal structure (molecular packing) they are polymorphic modifications or polymorphs.

**Solvates:** Also known as pseudo-polymorphs, they are crystalline solid adducts containing solvent molecules within crystal structure, in either stoichiometric or non-stoichiometric proportions (Vippagunta et al., 2001).

A single molecule in its solid state can exhibit several physical forms (polymorphs, solvates or amorphous). The kind of form in which it exists primarily depends on the molecular structure (functional groups in a molecule) and secondarily on the method by which it is prepared.
1.1.2 Forces responsible for crystal packing

*Organic crystals* are held together mainly due to non-covalent bonds as compared to ionic linkages in the *Ionic crystals* (Byrn et al., 1999). The non-covalent linkages are either hydrogen bonds or non-covalent attractive interactions. The hydrogen bond and the non-covalent attractive interactions result in regular arrangement of molecules in the crystal. The non-covalent attractive interactions (non-bonded interactions) depend on the dipole moment, polarizability and electronic distribution of the molecule. The hydrogen bonding interactions require hydrogen donor and acceptor functional groups in the molecule.

Crystal packing also depends on symmetry or lack of symmetry of the molecule. The molecular symmetry decides how molecules are packed in the crystal and also sometimes decides the symmetry of crystals. When a single molecule can exhibit many forms, the relative stability and hence the predominance of a particular form can be determined using the *Density rule*, which states that 'if one modification of a molecular crystal has lower density than another, it may be assumed to be less stable at absolute zero' (Burger and Ramberger, 1979). A higher density means more close packing of the crystals and smaller free energy. It follows that, smaller is the bond length stronger will be the intermolecular/intramolecular bonds holding the molecules in the crystal for denser polymorphs, hence will exhibit a higher melting temperature and fusion enthalpy. There are a few exceptions reported in the case of pharmaceuticals e.g. Indometacin stable γ-form has lower density (1.37 gm/cc) than the meta-stable α-form (1.43 gm/cc) (Chen et al., 2002).

1.1.3 Pharmaceutical process influenced by the physical form

Importantly, each polymorphic form will exhibit distinct physicochemical properties (Table 1-1) which can ultimately affect the pharmaceutically important processes listed in Table 1-2.

<table>
<thead>
<tr>
<th>Density</th>
<th>Hardness</th>
<th>Cleavage/plasticity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Melting point, enthalpy</td>
<td>Chemical/physical</td>
<td>Solubility</td>
</tr>
<tr>
<td>(thermodynamic properties)</td>
<td>stability</td>
<td></td>
</tr>
<tr>
<td>Optical properties</td>
<td>Water uptake</td>
<td></td>
</tr>
</tbody>
</table>

*Table 1-1:* The list of properties that are dependent on the physical form of a compound (Byrn et al., 1999).
Table 1-2: A list of pharmaceutical processes influenced by the physical form of a compound (Byrn et al., 1999).

The importance of polymorphic form in pharmaceutical development is due to their differences in solubility, mechanical properties and physical stability.

1.1.4 Pharmaceutical implications of the physical form

<table>
<thead>
<tr>
<th>Purpose</th>
<th>Substance</th>
<th>Crystal form</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stable</td>
<td>Beclomethasone</td>
<td>Chlorofluorocarbon solvate</td>
</tr>
<tr>
<td>Non-hygroscopic</td>
<td>Amoxicillin</td>
<td>Anhydrous sodium salt</td>
</tr>
<tr>
<td></td>
<td>Azithromycin</td>
<td>Dihydrate</td>
</tr>
<tr>
<td>High water solubility</td>
<td>DDI, DDT</td>
<td>Monohydrate</td>
</tr>
<tr>
<td>High bioavailability</td>
<td>Mefloquine HCl</td>
<td>Polymorph</td>
</tr>
<tr>
<td>High activity</td>
<td>Cefuroxime axetil</td>
<td>Amorphous</td>
</tr>
<tr>
<td>Improved flow</td>
<td>Ibuprofen</td>
<td>Crystalline</td>
</tr>
<tr>
<td></td>
<td>Piroxicam</td>
<td>β-form</td>
</tr>
<tr>
<td>Improved tabletting</td>
<td>Sorbitol</td>
<td>γ-form</td>
</tr>
</tbody>
</table>

Table 1-3: Applications of various crystal forms (Byrn et al., 1999).

The fact that different physical forms of the pharmaceuticals exhibit different pharmaceutical properties makes the selection of appropriate physical form inevitable. A review of various physical forms patented for particular pharmaceutical applications (Byrn et al., 1999) indicates that a particular physical form is selected over other for either one of the following advantages – improved stability, non-hygroscopic, injectable, immediate release, highly water soluble, improved flow, improved tabletting etc. (examples in Table 1-3). The amorphous form of an active is particularly important for improvement in dissolution properties, which is an issue in the case of many hydrophobic drug molecules. The amorphous state of an active is used in formulation of many solid dosage formulations e.g. Spectrace® (Cefditoren Pivoxil), Crestor® (Rosuvastatin calcium) and Ceftin® (Cefuroxime axetil) etc. with the main advantage of improved bioavailability. Although the amorphous state exhibits
improved dissolution properties it is the least favourite solid state because of its instability and tendency to transform into the crystalline state. There are several reviews which discuss the issues related to generation, characterisation and the significance of the amorphous state in the pharmaceutical arena (e.g. Craig et al., 1999; Hancock and Zografi, 1997; Yu, 2001) which emphasize the importance in understanding the amorphous state. The amorphous state has not only been the subject in pharmaceuticals but also in many other braches broadly characterised as the material sciences (Angell, 1995).

The amorphous state may have faster dissolution rate (which is many times mistaken as higher solubility) sometimes better flow properties and compression characteristics than the corresponding crystalline form. Apart from those advantages the amorphous state may be encountered due to various pharmaceutical processes such as spray drying, freeze drying and milling. Whatever the reason for generation of the amorphous state, it is always important to better understand it.
1.2 Amorphous – lack of morphology

The amorphous state of matter has been attracting attention of scientists from various fields. Physicists, ceramicists, metallurgists, polymer and material scientists have studied the amorphous state for various reasons. The amorphous state has a variety of applications and is used in plenty of applications of daily use; the most noteworthy is glass.

Three dimensional long-range order, present in the crystal state is absent in the amorphous state and the position of molecules is random to one another as in a liquid (Figure 1-1); hence it can be defined as, a liquid that has lost its ability to flow. In liquids, the molecules have translational, rotational and vibrational degrees of freedom. In the case of crystalline solids translational and rotational degrees are present to a lesser extent or absent due to rigid arrangement of molecules (molecules can only vibrate to some extent). Whereas, in amorphous state molecules can vibrate and rotate just like in liquid state (even though these degrees of freedom depend on the individual molecule and temperature). This means that molecules in the amorphous state exhibit more internal energy as compared to the crystalline state (Imaizumi et al., 1980).

![Figure 1-1: Molecular arrangement in amorphous and crystalline state.](image)

The amorphous state is also known as a glassy state, because of its low refractive index as compared to the crystalline state. Angell (Angell, 1996) has defined the glassy state as ‘any liquid with sluggish crystallisation kinetics becoming structurally arrested over a finite range of temperature’.
1.3 Thermodynamics of the glass formation and generation of amorphous state

![Diagram showing Enthalpy, Entropy, Volume vs Temperature with phases Liquid, Super-cooled liquid, Glass 1, Glass 2, Crystal and corresponding temperatures $T_k$, $T_g$, $T_{g1}$, $T_{g2}$, $T_m$.]

**Figure 1-2:** The process of glass transition observed by cooling a liquid melt; the type of glass formed (glass 1 and glass 2) depends on the cooling rate, indicating that $T_g$ of the glass formed would be higher when it is cooled faster. $T_k$ is Kauzmann temperature or the least possible $T_g$ when cooled at infinitesimally slow rate, it is the hypothetical temperature used as zero mobility temperature. Reproduced from (Ediger et al., 1996).

The process of glass formation by quench cooling of a liquid melt is explained thermodynamically in Figure 1-2. When a liquid melt is cooled down below melting temperature, a super-cooled liquid state is entered provided that the crystallisation event is avoided. A substance should exist as a solid below its melting point (or should crystallise upon cooling below melting point), but a few substances (those which can form a glass) continue to be liquid like and hence are described as super-cooled liquids. Further cooling of a super-cooled liquid causes a continuous loss in enthalpy or in other properties over a certain temperature range, after which, a break in temperature dependence of enthalpy is seen. A continuation of cooling beyond the break point does not lead to concomitant loss in enthalpy values, or some of the enthalpy is frozen in at this stage. A break in enthalpy versus temperature relationship is also accompanied with a sharp rise in viscosity values (viscosity similar
to the solid state). This phenomenon of transition from liquid like to a solid like state (or a sudden viscous slowdown) on cooling of the melt is called the glass transition. There are several theories explaining why a glassy state is formed and the observed characteristics of the glassy state but none of them can successfully explain all the properties of the observed glass. The theories are summarised in an excellent review (Ediger et al., 1996). The most widely used theory is mode coupling theory (MCT) of viscous slowdown. As the name indicates some modes of molecular relaxation separate as the super-cooled liquid state is cooled down below a certain temperature.
1.4 Methods for generation of amorphous state

The amorphous character can be generated intentionally (to explore the advantages of amorphous state e.g. solubility etc.) or unintentionally during the handling and the processing of pharmaceuticals (e.g. milling). The different methods which can potentially generate amorphousness have been discussed in detail (Angell, 1995; Hancock and Zografi, 1997) and can be summarized as follows,

1.4.1 Super-cooling of liquid melt

This is the most widely used method for generation of the amorphous state. A solid is melted and then rapidly cooled down, to avoid the crystallisation process. The liquid melt enters into a super-cooled liquid state and then into a glassy state in which molecules are arrested kinetically in amorphous state. The method could be depicted thermodynamically in Figure 1-2. This method is used industrially as a melt extrusion process (Albano et al., 2002) or spray quenching (spraying liquid melt into liquid nitrogen or chilled water).

1.4.2 Solvent evaporation

A solid crystalline sample is dissolved into a solvent, which is then evaporated rapidly leaving amorphous solids behind. The removal of solvent could be performed industrially by a freeze drying method. The solvent removal could also be performed by employing the method of spray drying. The process of freeze drying involves extended drying at primary and then secondary drying temperatures. The freeze drying procedure may cause the amorphous state to anneal and can induce crystallisation.

1.4.3 Milling or micronization

The process of milling or micronization is primarily used for particle size reduction (Figure 1-3) in pharmaceutical processing (Williams et al., 1999). The particle size is not only important for formulation of dry powder inhalers (Steckel et al., 2003) but also governs the dissolution rate. Albeit, these processes often induce undesired amorphous character or disordered regions in the crystalline sample, these disordered regions are mainly located at the surface of crystalline powder (Newell et al., 2001a). Theoretically the milling process activates powder surface by supplying mechanical energy and this activation may induce the disorders in the crystalline sample, these disorders may be retained in the solid state and ultimately influence the properties of
a finished product. Although it is also possible that the generated amorphous state is short lived but it may not revert back to the original crystalline state.

**Figure 1-3:** Scanning electron micrograph of salbutamol sulphate a. Unprocessed crystals, b. Agglomerated fine particles or amorphous regions after ball milling from (Brodka-Pfeiffer et al., 2003).

Various approaches have been reported to stabilize the disordered state produced by milling e.g. cryogenic grinding (Crowley and Zografi, 2002) of crystalline indomethacin, which involves immersion of the milling vessel in liquid nitrogen to stabilize the disordered regions. Other approaches may include co-grinding with silicone dioxide SiO$_2$ and Mg(OH)$_2$ (Watanabe et al., 2002) and recently with Neusilin (amorphous magnesium aluminosilicate) (Gupta et al., 2003). The milling has been used in generating highly amorphous form (>95%) of cefuroxime axetil but required extended milling with either one of the excipients such as silica, talc, sodium chloride, calcium carbonate or mixtures thereof (Somani et al., 2000)

### 1.4.4 Precipitation from solution

When a solution is added to an anti-solvent (which is miscible with the solvent) the solute may precipitate as amorphous solid. The precipitation method is employed very effectively to manufacture the amorphous form of Cefuroxime axetil (Karimian et al., 1998).

### 1.4.5 Dehydration of hydrates

These are the solid state diffusion controlled reactions, during this process the solvent molecules, which are part of the crystal lattice are removed. The removal of solvent molecules leaves a skeletal which collapses into amorphous state. This technique has been reported for the formation of amorphous state from a dihydrate of Carbamazepine (Li et al., 2000).
1.4.6 Compaction or compression of the crystals

Normally crystals are extremely brittle and lack elasticity. When crystalline solid is subjected to compression, crystal faces with weak, brittle bonding fracture and form a denser amorphous state. These transformations may be undesirable since the tablet properties are influenced by the solid state properties of an active ingredient. Many times these tablets exhibit undesirable effects such as capping or cracking associated with the release of compression stress.
1.5 Glass forming tendency and molecular conformational flexibility

![Image showing molecular conformations of sorbitol (top) and D-mannitol (bottom), in the free (liquid) state (left) and the crystalline state (right).](image)

**Figure 1.4:** Molecular conformations of sorbitol (top) and D-mannitol (bottom), in the free (liquid) state (left) and the crystalline state (right). In the case of sorbitol, the molecular conformation remains the same in both states, whereas in the case of D-mannitol different conformations could be seen in the liquid and the solid state (see text for more details).

Not all molecules can form the amorphous state readily or they may not form the amorphous state at all. The formation of amorphous glass is mainly influenced by thermodynamic or kinetic properties of the molecule and may be very straightforward for some (good glass formers) but difficult for other molecules (poor glass formers). Thermodynamically if a crystalline state is not very different energetically, then the amorphous state is formed readily. Whereas kinetically a slow crystallisation rate may allow the material to be frozen in a glassy state (Yu, 2001).

The issue which needs further attention in glass formation tendency and the stability of a glass is the conformational flexibility of molecule (Yu et al., 2000). A molecule can exhibit different conformations and configurations. Most of the pharmaceuticals are organics containing aromatic benzene rings with substitutions. An aromatic ring with substitutions is a highly strained structure and can exhibit different conformations but the most stable are boat and chair forms. In the case of a conformationally flexible molecule, a number of conformations are possible and molecules in a solution or in a molten state can be present as a mixture of various conformations. But in a crystalline state conformational flexibility is restricted and molecules are held together with specific conformation and configuration in a fixed cage. This means the process of crystallisation from a solution or a melt proceeds with the selection of a particular conformer. The process is particularly slow for a conformationally flexible molecule (which can be present in various conformations at a time) and molecules can easily enter into a glassy amorphous state. A number of stable configurations a molecule can exhibit can be determined using molecular modelling software; hence such
software can be used effectively in estimating a glass forming capability of the molecule.

This concept can be exemplified using different crystallizing tendency of hexitols (Siniti et al., 1993b; Siniti et al., 1993a). Sorbitol and iditol exhibit similar conformations in the free and crystalline states, whereas dulcitol and D-mannitol exhibit different conformations in the crystalline state as compared to the solution state (Siniti et al., 1999). These differences are depicted in poor glass formation ability of dulcitol and D-mannitol, whereas a stable glass for sorbitol and iditol.

Although this issue is not widely reported in the pharmaceutical literature, the use of particular enantiomers used in glass formation is mentioned in the case of Cefuroxime axetil (R and S) (Crisp and Clayton, 1991; Crisp and Clayton, 1985).
1.6 Properties of the amorphous state

![Graph showing temperature-dependent properties of a glass former]

---

**Figure 1-5**: The different domains of amorphous state obtained by plotting temperature dependent property of a glass former (enthalpy or volume); the curved region indicating a glass transition, reproduced from (Angell et al., 2000).

The state of matter below its melting point and lacking the long range order of molecules is classified as the amorphous state. By this definition a glassy state (below Tg) along with super-cooled liquid state (above Tg) could be classified as the amorphous state. A few workers describe super-cooled liquid state as a rubbery state. The glass transition temperature (Tg) distinguishes glassy and rubbery state (Figure 1-5). The transition from a super-cooled liquid state to a glassy state or vice versa is accompanied with change in many properties. The amorphous state could be studied by dividing various modes it exhibits with respect to glass transition into three domains as follows (Figure 1-5) (Angell et al., 2000).

### 1.6.1 Glass-former in internal equilibrium (Domain A)

This domain covers the glass former in super-cooled liquid region, where temperature is significantly higher than the glass transition temperature. A pharmaceutical scientist seldom experiences this state or this temperature domain (except in the processes such as extrusion, and sometimes in the freeze drying or spray drying etc.) but it would be interesting to exploit this state in order to understand the glassy state (domain C). It is characterised by measuring mainly the transport related properties (e.g. viscosity, diffusivity, conductivity etc.) or relaxation time.
The super-cooled liquid state (Domain A) is described as an equilibrium state, as a small change in temperature leads to almost instantaneous change in temperature related properties; hence this state is devoid of any time dependent properties.

**Figure 1-6:** Viscosity as a function of reduced inverse temperature for three super-cooled liquids of SiO$_2$, glycerol and o-terphenyl (in Domain A). Reorientation time ($\tau$) shown for o-terphenyl (OTP) only. Reproduced from (Ediger et al., 1996).

Typical properties of super-cooled liquid can be seen from Figure 1-6. The viscosity in Domain A could be compared with the typical viscosity of water or benzene at room temperature ($10^{-2}$ P). The temperature dependence of viscosity for SiO$_2$ super-cooled liquid showed a near Arrhenius dependence (linear relationship between log $\eta$ and $1/T$), whereas for glycerol it was fairly non-Arrhenius response which, shows extreme non-linearity for o-terphenyl. The reorientation time for o-terphenyl was very small in this domain (ranging from picoseconds to nanoseconds) (Chang et al., 1994). For o-terphenyl temperature dependence of viscosity followed the same relationship as temperature dependence of reorientation time. Most of the super-cooled liquid states fall in two extremes of SiO$_2$ and o-terphenyl. On the basis of these properties super-cooled liquids have been classified as strong or fragile (Angell, 1991). The strong liquids follow Arrhenius relationship for temperature dependence of relaxation process and viscosity. The strong liquids are characterised by three dimensional network structures of covalent bonds (e.g. SiO$_2$). The fragile liquids exhibit non-Arrhenius
relationship and consist of molecules interacting through non-directional, non-covalent interactions like dispersion forces (e.g. o-terphenyl). The properties of a glass (below glass transition temperature, Domain B) need not be a continuation of properties observed in super-cooled liquid domain.

A non-Arrhenius (or non-exponential) temperature dependence of properties in the super-cooled liquid domain is approximated with Vogel-Tamman-Fulcher equation (VFT) (Equation 1).

$$\tau = \tau_0 \exp\left(\frac{B}{T - T_\infty}\right)$$

Equation 1

Where, $\tau$ is relaxation time and could be replaced by viscosity, $\tau_0$ is the shortest possible relaxation time, $T_\infty$ is the temperature of longest relaxation time and $B$ is a material parameter related to its fragility. Although widely used, applicability of equation 1 is always questioned near the glass transition temperature (Tg).

### 1.6.2 Glass former around Tg: time dependent properties (Domain B)

The glass formers in the region of Tg demonstrate time dependent properties. This means that material properties are a function of both temperature as well as time. As shown in Figure 1-5 this domain spans from just above Tg (super-cooled liquid) through to the glassy domain (slightly below Tg). This domain can be further divided into two sub-domains. First a region slightly above glass transition temperature, the super-cooled liquid domain and second below glass transition temperature. As opposed to domain A where viscosity is comparable to liquid water domain B is highly viscous ($10^{13}$ P), the viscosity further increases in the glassy region. The molecular relaxation time (near Tg) reaches a high value from seconds to years (depending on the nature of glass) as compared to picoseconds in the super-cooled liquid domain. These slow molecular relaxation processes give rise to time dependent properties (e.g. structural relaxation of glass or aging and annealing). This domain is of particular interest to the pharmaceutical scientist as most of the pharmaceutical glasses are handled in this region. A glass can undergo both structural and bulk relaxation above Tg, where as below Tg structure remains almost constant while bulk relaxations still persist.

### 1.6.3 Frozen glass former (Domain C)

Below a certain temperature the primary relaxation processes, or \(\alpha\)-relaxation processes in glass are frozen described as Domain C (Figure 1-5). The \(\alpha\)-relaxations are mainly due to molecular rotations which are coupled together. This implies only
decoupled motions are possible in the Domain C. This domain is of particular interest to pharmaceutical applications where temperature conditions for the handling of amorphous state are important. Although there are reports stating that the dielectric relaxations are detected in this domain, particularly in ionic glasses (e.g. Na⁺, K⁺ glasses and metallic glasses).

1.7 Techniques for the characterisation of amorphous solids

The amorphous state has been studied by numerous techniques which include calorimetric analysis and various spectroscopic techniques. The easiest way to characterise the amorphous state is by microscopic observation (lack of birefringence). The detection of amorphous state in pharmaceuticals has been mainly focused on the presence of partially amorphous solids (ordered and disordered structures present together) requiring quantification of the ordered and the disordered structure.

Among the various techniques used most commonly, DSC (by heat of crystallisation) and XRD (Saleki-Gerhardt et al., 1994) have been reported to detect amorphous state to only more than 10% of crystalline or ordered state. The other techniques (Table 1-4) with much lower detection limits reported are MDSC 1-3% (Guinot and Leveiller, 1999), water sorption (DVS) 0.05% (Buckton and Darcy, 1995), solution calorimetry 0.5% w/w (Hogan and Buckton, 2000; Pikal et al., 1978), isothermal microcalorimetry 1% (Briggner et al., 1994) and <1% for hydrophobic drugs (Ahmed et al., 1996), NIR and DVS combination 1% (Hogan and Buckton, 2001), FTIR 1% (Taylor and Zografi, 1998a), Hyper-DSC 1.0% (Saunders et al., 2004) and thermally stimulated current spectroscopy 1-2% (Venkatesh et al., 2001).

Most of the above mentioned techniques are based on two state model of a partially disordered state (coexistence of 100%crystalline and 100% amorphous regions). The presence of rigid amorphous regions along with mobile amorphous and crystalline regions may lead to erroneous quantification of amorphousness (Craig et al., 2001). Although, the presence of rigid amorphous state is possible theoretically this has not yet been proven for low molecular weight pharmaceuticals.

The amorphous state generated mainly at the surface of a particle during milling or micronization has been distinguished by inverse phase gas chromatography (IGC) (Newell et al., 2001a) and by microthermal analysis (Craig et al., 2002).
<table>
<thead>
<tr>
<th>Technique</th>
<th>Distinct Characteristic of the amorphous state</th>
<th>Lower limit of detection</th>
</tr>
</thead>
<tbody>
<tr>
<td>DSC</td>
<td>Glass transition by step up in the Cp value, Heat of recrystallisation</td>
<td>10%</td>
</tr>
<tr>
<td>Hyper-DSC</td>
<td>Amplification of the ΔCp response at Tg</td>
<td>1%</td>
</tr>
<tr>
<td>MDSC</td>
<td>Separation of Cp and relaxation heat flow at Tg, recrystallisation</td>
<td>1-3%</td>
</tr>
<tr>
<td>Isothermal Microcalorimetry</td>
<td>Heat of isothermal recrystallisation</td>
<td>1%</td>
</tr>
<tr>
<td>Ampoule</td>
<td>Heat solvation and lattice bond energy</td>
<td>0.5%</td>
</tr>
<tr>
<td>Solution</td>
<td></td>
<td></td>
</tr>
<tr>
<td>XRD</td>
<td>Lack of sharp diffraction pattern</td>
<td>5-10%</td>
</tr>
<tr>
<td>NIR</td>
<td>Broadening of the bands along with the shift in wavelength</td>
<td>1%</td>
</tr>
<tr>
<td>FTIR</td>
<td>Broad vibrational band</td>
<td>1%</td>
</tr>
<tr>
<td>Raman</td>
<td>Broad vibrational band</td>
<td>--</td>
</tr>
<tr>
<td>FTIR</td>
<td></td>
<td>--</td>
</tr>
<tr>
<td>DVS</td>
<td>Water uptake by amorphousness</td>
<td>0.2%</td>
</tr>
<tr>
<td>Solid state NMR</td>
<td>Broad peak</td>
<td>--</td>
</tr>
<tr>
<td>Viscometry</td>
<td>Sudden decrease in the viscosity above Tg</td>
<td>--</td>
</tr>
<tr>
<td>DMA</td>
<td>Loss of mechanical properties above Tg</td>
<td>--</td>
</tr>
<tr>
<td>Dielectric analysis</td>
<td>Dielectric loss at glass transition</td>
<td>--</td>
</tr>
<tr>
<td>TSC</td>
<td>Depolarization current due to molecular relaxations</td>
<td>1-2%</td>
</tr>
<tr>
<td>Microscopy</td>
<td>Absence of birefringence</td>
<td>--</td>
</tr>
<tr>
<td>Density</td>
<td>Lower density than the crystalline state</td>
<td>--</td>
</tr>
</tbody>
</table>

Table 1-4: A list of techniques used for the characterisation of the amorphous state with their lower limit of detection.
1.8 Comparison of glass formed by different routes

Several methods are discussed in section 1.4 for generation of the amorphous state. Although all the techniques are aimed at reducing the long range order of the crystalline state into a short range or no order at all of the amorphous state they differ in their efficiency. Hence the amorphous state formed by different methods could inevitably exhibit different characteristics such as glass transition temperature (Tg), water uptake profile (hygroscopicity), aging or enthalpy recovery, surface characters, morphology, the extent of short range order and finally the crystallisation tendencies. The extent of short range order could be studied by powder X-ray diffraction (XRPD) and heat of solution. It was demonstrated for β-lactam antibiotics that the amorphous state generated was more ordered in the case of spray drying as compared to freeze drying (Pikal et al., 1978). In another comprehensive study amorphous trehalose was produced by freeze drying, spray drying, dehydration and melt quenching (Surana et al., 2004b). It was observed that although Tg remained almost constant, other properties such as enthalpy relaxation, water uptake profile, crystallisation tendencies and morphological features varied with the method used to generate the amorphous state. The amorphous state produced by dehydration showed the highest enthalpic recovery at Tg and also the highest tendency to recrystallise. This was opposed to the melt quench technique which produced the amorphous state with the least or no crystallisation tendency. The rate of water uptake was the highest with spray dried solid as compared to other methods.

It is inevitable that the clear differences could be observed in the amorphous state produced using different methods. This initiates the highly debated question about what should be used as a standard for quantitative estimation of the range order in the amorphous state.
1.9 Molecular mobility, molecular relaxation and crystallisation in amorphous state

Figure 1-7: A comparison of various relaxation times measured for o-terphenyl. \(\alpha\)-relaxation: dielectric relaxation (•), NMR(●); \(\beta_s\)-relaxation: dielectric relaxation (○); \(\beta_l\)-relaxation (x). Reproduced from (Ediger et al., 1996).

As explained in section 1.6 molecules in the amorphous state have different degrees of freedom (or mobility) in the glassy and the super-cooled liquid state. In the super-cooled liquid state, which is more similar to the liquid state, molecules can rotate, vibrate and even translocate. These molecular motions in the amorphous state are often linked to the molecular relaxations. The time taken for a particular motion to take place can be defined as the relaxation time (Angell, 1995). A molecule can exhibit different kinds of relaxations at a time depending on the nature of a glassy state. These relaxations are often coupled especially in the super-cooled liquid state, which means that the molecular motion is observed as a co-operative motion.

Molecular motions in the super-cooled state are particularly faster i.e. they are observed on a shorter time scale (10^{-9} sec. to 10^2 sec.) or they have shorter relaxation times, whereas in a glassy state (below \(T_g\), which is more similar to solid state) relaxation times are much longer (few minutes to years). Molecular relaxations in the super-cooled liquid state are often classified (Ediger et al., 1996) as primary or slow relaxations (\(\alpha\)-processes) and secondary relaxations (\(\beta\)-processes). \(\alpha\)-processes roughly correspond to molecular rotations. \(\beta\)-processes occur on a shorter time scale.
as compared to \( \alpha \)-processes (Figure 1-7) and are further classified as \( \beta \)-slow (\( \beta_a \)) and \( \beta \)-fast processes (\( \beta_f \)). \( \beta_a \)-processes are due to partial reorientation of molecules whereas \( \beta_f \)-processes are believed to be due to complex collective an-harmonic cage rattling processes. Variation of time scales of theses processes with temperature is shown in Figure 1-7. Glass transition as seen from the step change in heat capacity using DSC is often been linked with \( \alpha \)-relaxations.

The internal energy of amorphous state is higher than crystalline state (Figure 1-2, section 1.3) and it follows that the molecules in amorphous state are more mobile (kinetically). A higher molecular mobility has often been linked to the crystallisation tendency of amorphous state (Aso et al., 2004; Aso et al., 2000). Crystallisation rate \( k \) for the amorphous state at temperature \( T \) is often written simply as equation 2

\[
k = D(T)f(T) \tag{Equation 2}
\]

Where, \( D(T) \) represents a temperature dependence of molecular diffusion across the nuclear amorphous matrix interface and \( f(T) \) describes a nucleation free energy term. Assuming a temperature dependence of term \( D(T) \) is much larger than \( f(T) \), crystallisation rate has been correlated to molecular relaxation time of the amorphous state as equation 3

\[
\frac{k}{k_{Tg}} \equiv \frac{D_T}{D_{Tg}} \equiv \left( \frac{T}{T_{Tg}} \right) \left( \frac{\eta}{\eta_{Tg}} \right)^\xi = \left( \frac{T}{T_{Tg}} \right) \left( \frac{T_{Tg}}{\tau} \right)^\xi \tag{Equation 3}
\]

Where, \( D_T, \eta \) and \( \tau \) are diffusivity, viscosity and molecular relaxation time at temperature \( T \), while \( D_{Tg}, \eta_{Tg} \) and \( T_{Tg} \) at temperature \( T_{Tg} \) respectively.

Molecular mobility is measured by various approaches which could be summarized as follows,

1.9.1 Molecular mobility using the empirical Kohlrausch-Williams-Watts equation

This approach is based on measuring the rate of relaxation of the amorphous state by aging it isothermally. Molecular mobility or relaxation times are then calculated by estimating the extent of relaxation at time \( 't' \) using the empirical Kohlrausch-Williams-Watts equation (equation 4) (Williams and Watts, 1970).
\[ \Phi(t) = \exp \left[ -\left( \frac{t}{\tau} \right)^\beta \right] \] \hspace{1cm} \text{Equation 4}

Where, \( \Phi(t) \) is the extent of relaxation obtained by annealing amorphous solid isothermally for time \( t \), \( \tau \) is the average molecular relaxation time and \( \beta \) is a parameter related to distribution of molecular relaxation times.

The extent of relaxation can be estimated by measuring volume relaxations or dielectric relaxations, although the most commonly used parameter is enthalpy relaxations \( \Delta H \) (Hancock et al., 1995). When a glass ages it relaxes towards the equilibrium glassy state and loses heat; the enthalpy relaxation is observed as an equivalent heat absorbed (to the heat lost during aging) as an endothermic response along with glass transition during a subsequent heating scan.

The extent of relaxation \( \Phi(t) \) is calculated from enthalpy relaxation \( \Delta H(t) \) at time \( t \) using equation 5. The maximum possible value of enthalpy relaxation \( \Delta H_{(o)} \) could be calculated using equation 6.

\[ \Phi(t) = 1 - \left( \frac{\Delta H(t)}{\Delta H_{(o)}} \right) \] \hspace{1cm} \text{Equation 5}

\[ \Delta H_{(o)} = (T_g - T) \Delta C_p \] \hspace{1cm} \text{Equation 6}

Where \( T_g \) and \( T \) are glass transition and aging temperature respectively, \( \Delta C_p \) being the specific heat change during glass transition.

The enthalpy relaxation can be measured calorimetrically either by using a differential scanning calorimeter (DSC) as explained before, or isothermally by directly following the heat loss using a Thermal Activity Monitor (TAM) (Liu et al., 2002).

### 1.9.2 Molecular mobility from the Adam-Gibbs-Vogel (AGV) equation (heating rate dependence of glass transition)

This approach is originally based on Arrhenious behaviour (equation 7) for temperature dependence of molecular relaxation time (Ediger et al., 1996).
\[
\tau(T) = \tau_0 \exp \left( \frac{\Delta H}{RT} \right)
\]

Equation 7

Where \( \tau(T) \) is the molecular relaxation time at temperature \( T \), \( \tau_0 \) is the shortest possible relaxation time, \( \Delta H \) is the activation energy and \( R \) is the gas constant. Limitations of equation 7 (as observed by changes in apparent activation energy \( \Delta H \)) are overcome by using the Adam-Gibbs-Vogel (AGV) equation (equation 8) (Aso et al., 2001).

\[
\tau(T) = \tau_0 \exp \left( \frac{ST_0}{T \left(1 - \frac{T_0}{T_f}\right)} \right)
\]

Equation 8

Where, \( \tau_f \) and \( \tau_0 \) are similar to equation 7, \( S \) and \( T_0 \) are parameters related to the strength and temperature corresponding to maximum relaxation time respectively and \( T_f \) is the fictive temperature. The calculation of parameters in equation 8, i.e. \( S \), \( T_0 \) and \( T_f \) was explained in detail before (Andronis and Zografi, 1998; Aso et al., 2001). The calculation of \( S \) and \( T_0 \) is based on heating rate dependence of glass transition. The parameters \( S \) and \( T_0 \), obtained are mainly used for comparing the strength of and zero mobility temperature \( (T_0=T_k) \) of amorphous glass respectively. \( T_k \) is known as the Kauzmann temperature where the molecular mobility in the amorphous state theoretically ceases, it is also known as a glass transition temperature obtained with the slowest possible cooling rate (Ediger et al., 1996). In the pharmaceutical arena \( T_k \) is used as a temperature for the least possible crystallisation rate (Hancock et al., 1998a).

### 1.9.3 Molecular mobility by the Cole-Davidson equation

This approach is based on estimation of molecular mobility in the super-cooled liquid region by measuring the dielectric constant as a function of frequency using the Cole-Davidson equation (equation 9) (Lindsey and Patterson, 1980) as follows,

\[
\frac{\varepsilon^*(\omega) - \varepsilon_\infty}{\varepsilon_0 - \varepsilon_\infty} = \frac{1}{(1 + i\omega\tau_{CD})^{\beta}}
\]

Equation 9

Where, \( \varepsilon^*(\omega) \) is a complex frequency-dependant dielectric constant, \( \varepsilon_\infty \) is the high frequency limiting value of the real part of the dielectric constant, \( \varepsilon_0 \) is the low frequency limiting value of the real part of the dielectric constant, \( \tau_{CD} \) is the maximum relaxation time for a material under study and \( \beta \) is the width parameter \((0<\beta<1)\).
Molecular mobility at temperature $T$ can be calculated using equation 9 and the molecular mobility in a glassy state can be estimated using the VTF equation (equation 1). Molecular mobility thus obtained can be defined as the dielectric relaxation time.

This approach has been used in calculation of molecular mobility for amorphous indomethacin (Andronis and Zografi, 1998). Shear modulus data (obtained from dynamic mechanical analysis) can be used with the empirical Cole-Davidson equation (modified Cole-Davidson equation) to measure shear relaxation time (Andronis and Zografi, 1997).

1.9.4 Molecular mobility by thermally stimulated current

In this method amorphous sample is heated above its glass transition temperature and a fixed magnetic field is applied which, causes orientation of the molecular dipole. The magnetic field is then removed and sample is cooled well below its glass transition temperature. In a last step the sample is heated in a linear ramp and the current generated due to depolarization (due to relaxation process of magnetic dipole) is detected (Correia et al., 2001). Using the thermal window method with thermally stimulated current spectroscopy (TSC) it has been possible to resolve different molecular relaxation processes and study them individually. Relaxation time for a molecular motion under consideration is calculated from equation 10,

$$\tau(T) = \frac{1}{\int_{T}^{\infty} \frac{J(T')dT'}{J(T)}}$$  \hspace{1cm} \text{Equation 10}

Where, $\tau(T)$ is the molecular relaxation time and $J(T)$ is the depolarization current density at temperature $T$.

1.9.5 Molecular mobility by nuclear magnetic resonance (NMR)

This is one of the applications of solid state NMR (Pulsed NMR). In this technique a short burst of radiofrequency (Rf) power is supplied to a sample in a fixed magnetic field; causing the excitation of a certain nuclei. Rf power is then turned off and the net intensity of the magnetic field is then observed as excited nuclei return to their original equilibrium state with respect to their surroundings. This rate is defined as NMR relaxation time and can be observed for H\textsuperscript{1} or C\textsuperscript{13} nuclei.
1.10 A comparison of molecular mobility obtained using different methods

![Graph showing comparison of molecular mobility](image)

**Figure 1-8:** A comparison of relaxation time values reported for amorphous indomethacin using various methods. TSC-1 and TSC-2 are obtained at minimum and maximum temperature limits.

It is very important to understand the meaning of molecular relaxation time measured using different methods and how the values compare among the various methods. Amorphous indomethacin is the only compound for which the molecular relaxation times have been measured using almost all of the above mentioned methods. The molecular relaxation time ($\tau$) calculated by dynamic mechanical analysis (DMA) (Andronis and Zografi, 1997), dielectric analysis (DEA), heating rate dependence of $T_g$ (Andronis and Zografi, 1998) and thermally stimulated current (Correia et al., 2001) are compared in Figure 1-8. Values of $\log \tau$ obtained by different methods above the glass transition temperature are particularly short and are comparable. Below $T_g$, molecular relaxations become sluggish (longer $\tau$ values). Above $T_g$, $\tau$ values obtained by enthalpy relaxation matched with $\tau$ values from the thermally stimulated current method but differ significantly from the heating rate dependence method.

Although equation 3 can be used as a means of comparison between the molecular mobility and hence the crystallisation rates at two temperatures its use in estimation of shelf life of the amorphous state is questionable. Another issue in the use of molecular relaxation time for the interpretation of the crystallisation tendencies is a lack of complete understanding about which molecular motions lead to crystallisation
and hence are of relevance. As described before a molecule in a given environment can relax in many different ways and further they all have different temperature dependencies. One type of relaxation may predominate in a certain range of temperature, but may be entirely irrelevant to the crystallisation process.
1.11 Interaction of water vapour with amorphous state

The exposure of solids to various amounts of water is possible during different pharmaceutical processes (e.g. wet granulation, drying etc.) or during routine handling of the powder. The interaction between water molecules and amorphous solid state is very different as compared to that with the crystalline solid state. The sorbed water may not just influence the physical properties of the amorphous solid (e.g. brittleness, flow properties, crystallisation etc.) (Kontny and Mulska, 1989) but could also lead to chemical instability (Pikal et al., 1977). These issues can be studied by understanding the physicochemical nature of the interaction, the state of sorbed water in the amorphous solid and its plasticisation effect. This could be interesting in understanding the physicochemical stability of the amorphous solid in the presence of sorbed water.

1.11.1 The physicochemical nature of the interaction

The amorphous state, due to higher free volume than the crystalline state, can allow free access to small molecules like water almost throughout the bulk of the solid. When exposed to high RH water molecules can be adsorbed on to or absorbed in to the amorphous material. The amount of water sorbed depends on the RH to which the amorphous solid is exposed along with the physicochemical nature of the solid.

The physical interactions between the amorphous solid and water primarily involves lowering of the Tg (Oksanen and Zografi, 1990). The Tg lowering effect is also described as a plasticisation of the amorphous solid. The extent to which Tg is lowered depends on the amount of water taken into the amorphous solid. It follows that at certain amount of water content dry Tg (glass transition temperature of dry solid) would be lowered to ambient temperature where, the amorphous solid would then exist in a less viscous rubbery state. Further water uptake leads to collapse of the amorphous solid and ultimately transformation into a more stable crystalline state. The crystallisation of the amorphous solid due to absorbed water is accompanied with desorption of water or a weight loss.

The shape of the water sorption isotherm for the amorphous solid (before induction of crystallisation) is typically similar to the type-II (initial shoulder) or type-III BET isotherms (Hancock and Zografi, 1993). The sorption of water involves either formation of physical bonds with the adsorbent (mainly hydrogen bonds) or condensation at the surface. All these processes are essentially exothermic. This implies that the amount of water sorbed at any particular humidity should decrease
with increase in temperature. Similar findings are reported for various sugars (Hancock and Dalton, 1999) and for PVP (Figure 1-9) (Oksanen and Zografi, 1990).

![Water sorption isotherms for PVP at various temperatures](image)

**Figure 1-9:** Water sorption isotherms for PVP at various temperatures, from (Oksanen and Zografi, 1990).

The physical aging of a glass (structural relaxation) reduces the rate and the extent of water uptake by the amorphous solid at low RH (10-20%RH), whereas this effect is levelled off by higher water uptake at high RH (Surana et al., 2004a). Water sorption has also been observed to remove the effects of physical aging and thus caused the enthalpy recovery in an aged sample. This effect of water has been observed below the glass transition temperature and hence indicated a complex interaction between water and amorphous solids.

1.11.2 Mathematical models to describe water uptake behaviour of the amorphous solids

The water sorption isotherm is studied most commonly using a model equation designed by Brunauer-Emmett-Teller (BET) (Brunauer et al., 1938) (equation 11).

\[
W = \frac{W_m C_p \left( \frac{p}{p^0} \right)}{1 - \left( \frac{p}{p^0} \right) + \left( \frac{C_p}{p^0} \right)^{-n}}
\]

Equation 11
where, \( W \) is the mass of water sorbed per unit mass of solid, \( p/p^0 \) is the relative vapour pressure of water, \( W_m \) is a constant related to the weight of water for monolayer coverage of the solid and constant \( C_b \) is related to the overall free energy of adsorption.

Applicability of the BET equation for the water sorption behaviour of the amorphous solid is questionable. This is due to the unrealistic nature of the constants like \( W_m \) (as water molecules exhibit free access throughout the bulk of the sample) and \( C_b \) (value expected to change as the sorption proceeds) for amorphous solid.

Other models which are based on the solution theory, primarily developed for polymers but used with high success for amorphous pharmaceuticals (Hancock and Zografi, 1993; Zhang and Zografi, 2000) are the Flory-Huggins model (equation 12) and the Vrentas model (equation 13).

\[
\frac{p}{p^0} = \phi_1 \exp\left[\left(1 - \frac{1}{x}\right)\phi_2 + \chi \phi_2^2\right]
\]

\[\text{Equation 12}\]

\[
\frac{p}{p^0} = \phi_1 \exp\left[\left(1 - \frac{1}{x}\right)\phi_2 + \chi \phi_2^2\right] \exp f
\]

\[\text{Equation 13}\]

where,

\[
f = \frac{\left\{M_1w_2^2\left[C_p^g - C_p\right]\left(\frac{dT_{gm}}{dw_1}\right)\left(\frac{T}{T_{gm}}\right)^{-1}\right\}}{RT}
\]

\[\text{Equation 14}\]

\( p/p^0 \) is the partial pressure of the solvent (water), \( x \) is the ratio of molar volumes of solvent and polymer, \( \phi_1 \) and \( \phi_2 \) are volume fractions of solvent and amorphous solid, \( \chi \) is the solvent molecule and the solid interaction parameter, \( C_p^g \) and \( C_p \) are the specific heat of the mix (solid with sorbed water) in the glassy and the rubbery state respectively, \( M_1 \) is the molecular weight of the solvent and \( dT_{gm}/dw_1 \) is a change in \( T_g \) of the mix with varying water mass fraction \( w_1 \).

The Vrentas model (equation 13) is particularly modified for water sorption below the glass transition and it transforms into the Flory-Huggins model (equation 12) when \( T_g \) of the mixture drops to \( T \). The Flory-Huggins model is particularly suitable for describing water sorption in the rubbery state. The various parameters obtained from these models have been shown to be effective in understanding the physicochemical nature of amorphous solid - water interactions (Hancock and Zografi, 1993).
1.11.3 The plasticisation of amorphous solid

Plasticisation is the phenomena by which the glass transition of the amorphous state in dry conditions (measured by any of the techniques) is lowered by mixing it with a substance having a lower Tg. Small molecules (e.g. water, methanol, ethanol, acetone etc.) when mixed with dry amorphous material can lower the Tg of the dry amorphous solid. As the amorphous solid continues absorbing water, its Tg reduces and at one particular water content the Tg is reduced to the experimental temperature. At this water content the solid changes from the highly viscous glassy state to the low viscosity rubbery state. The reduced viscosity of the rubbery state offers less resistance to the further intake of water and may lead to a change in the shape of the water sorption isotherm above this transition (Oksanen and Zografi, 1990). Further reduction in viscosity may also lead to increased flow properties and collapse of the amorphous solid as reported for lactose (Newell et al., 2001b). These transitions observed as a result of increasing the amount of water uptake may be correlated to the transitions observed by increasing the temperature of the amorphous solid (te Booy et al., 1992).

The Tg lowering effect of water is explained with free volume theory and is modelled using the Gordon-Taylor equation (Gordon and Taylor, 1952) (equation 15).

\[
T_{g(mix)} = \phi_1 T_{g1} + \phi_2 T_{g2}
\]

Equation 15

where \(\phi_1\) and \(\phi_2\) are the volume fractions of the two components and \(T_{g1}\) and \(T_{g2}\) are the glass transition temperatures of the individual components of the amorphous mixture.

The equation 15 is based on the perfect volume additivity or the simple mixing rule and hence does not take into account any interaction between the two components. For practical purposes equation 15 can be modified as equation 16.

\[
T_{gmix} = \frac{\left[w_1 \cdot T_{g1} + \left(K \cdot w_2 \cdot T_{g2}\right)\right]}{w_1 + \left(K \cdot w_2\right)}
\]

Equation 16

where,

\[
K = \frac{\rho_1 \cdot T_{g1}}{\rho_2 \cdot T_{g2}}
\]

Equation 17
w is the weight fraction and ρ is the true density. The value of K in equation 16 can also be calculated as follows,

\[ K = \frac{\Delta C_p}{\Delta C_p^1} \]

Equation 18

where, \( \Delta C_p \) is the specific heat change at the glass transition.

**Figure 1-10:** The variation of Tg for PVP and cellulose with different water content, line indicating the value predicted using the Gordon Taylor equation (equation 16) (Hancock and Zografi, 1994).

The amorphous solids follow the Gordon Taylor relationship for the dependence of Tg on the amount of water sorbed (e.g. PVP); whereas some amorphous compounds show deviations from the predictions of the Gordon Taylor (GT) equation (e.g. Cellulose) (Hancock and Zografi, 1994) (Figure 1-10). A positive deviation indicates that the actual depression in Tg is more than that predicted using GT equation; this could be due to i) the presence of crystalline regions in the amorphous solid, ii) the localization of the sorbed water on the surface or poor water mobility in the amorphous solid, iii) a kind of interaction taking place between the amorphous solid and the sorbed water.

### 1.11.4 The state of sorbed water in amorphous solid

The state of sorbed water implies mobility of water molecules in the amorphous solid. This issue is particularly important in considering water-amorphous interaction since water in different states can be assumed to exhibit different properties and hence variable effect on the amorphous state. On a molecular level, the process of water sorption proceeds with formation of hydrogen bonds with the amorphous state. The easily accessible sites, which located on a surface of amorphous solid get covered by water molecules. Interaction at this level can be assumed to be between water-solid molecules only and no interaction between water-water molecules. Water molecules at this level of sorbed water can be described to be tightly bound water (Zografi and Kontry, 1986). This process is believed to continue till the amount of water reaches...
some critical value which is correlated to monolayer water content \((W_m)\) from BET equation (equation 11) and observed as a shoulder on the water sorption isotherm (Oksanen and Zografi, 1990). \(W_m\) has been regarded as the critical water content since many physical properties change significantly at this water content (Kontny and Mulski, 1989). It is also believed that another critical point exists which is a multiple of \(W_m\), beyond which water starts exhibiting solvent like properties. This point has been determined to be three times \(W_m\) for starch and five times \(W_m\) for microcrystalline cellulose (Hollenbeck et al., 1978).

Another possibility is that, the water sorption by amorphous solid is a continuous process or there could be no distinction between tightly bound and freely mobile water states. Since the amorphous state lacks rigid intermolecular bonds, sorption of the water may proceed with the replacement of weak intermolecular bonds with hydrogen bonds comprising water molecules. This may be accompanied with possible changes in solid state conformations of molecules resulting in appearance of additional water binding sites. This process which is conceptually similar to the solvent effect of water molecules may continue along with initial water sorption (below \(W_m\)). The state of water in the amorphous solid can be studied using different techniques as described in the next section.

**1.11.4.1 The enthalpy of sorption to study the state of sorbed water**

![Figure 1-11: Net differential heat of sorption (cal/gm) for water vapour by native potato starch at 20°C as a function of amount of water sorbed per mass of dry starch (Zografi and Kontny, 1986)](image-url)
Using isothermal calorimetry enthalpy change due to the sorption process ($\Delta H_{\text{sorption}}$) could be studied as the amorphous solid is exposed to various levels of %RH. The $\Delta H_{\text{sorption}}$ could be compared with the enthalpy change due to the condensation for water ($\Delta H_{\text{condensation}}$). The higher value for $\Delta H_{\text{sorption}}$ than $\Delta H_{\text{condensation}}$ indicates that the interaction is predominantly between solid-water rather than between the water-water molecules. A change in differential heat of sorption values indicates the change in thermodynamic state of water as sorption proceeds (Figure 1-11).

1.11.4.2 Thermal analytical techniques to study the state of sorbed water

Amorphous solid with sorbed water when cooled below 0°C should show a freezing response depending on the state of water; unbound water should freeze whereas water bound to the solid state is unable to freeze. Proteins have shown to take up 0.3-0.4 g/g dry weight without any freezing at low temperature. Whereas un-freezable water in starches range from 0.25-0.3 g/g which is 3-4 times $W_m$ indicating water molecules bound in structured layers around the surface (Zografi and Kontny, 1986).

1.11.4.3 Nuclear magnetic resonance to the study state of sorbed water

Using this method relaxation times of $H^1$ and $C^{13}$ were measured as explained in section 1.2.6.5. The measurements were performed at various levels of water. $H^1$-NMR studies for water absorbed onto PVP indicated that the rotational and the translational mobility of water molecules was hindered by the presence of the solid polymer backbone, hence the absorbed water was present in two or more states with different modes or time scales of motion (Oksanen and Zografi, 1993). The authors also concluded that the presence of tightly bound water at the level of water corresponding to $W_m$ was unlikely. At higher levels of water content water mobility increases but is always less mobile than the bulk water. In general it was concluded that the state of water depends on the complex interaction with the solid and mainly on the viscoelastic properties of the solid, since it is known that the sorbed water lowers the Tg of the solid and at Wg solid transform into the less viscous state as compared to the glassy state. A less viscous state above Tg may be assumed to pose more free access to the small molecules like water.
1.12 Solubility: the advantage of amorphous state

The process of solubilization includes breaking of bonds holding the solute molecules in the solid state followed by formation of bonds between solute and solvent molecules (Martin, 1993b). The amorphous state lacks the strong intermolecular bonds which are present in the crystal lattice, consequently the amorphous state should solubilize rapidly as compared to crystals (amorphous solids do not need heat of solvation). A saturation point (or super-saturation) will generally be reached faster with the amorphous state than with the equivalent crystalline state, provided sufficient solids are added. If solubilized molecules are not taken away from the solvent they may crystallise and precipitate into a more stable crystalline state and hence the equilibrium solubility will depend on the intrinsic solubility of the stable crystalline state. The process of dissolution of the amorphous and the crystalline state has been exemplified using the example of indomethacin in Figure 1-12.

![Graph](image)

**Figure 1-12**: The aqueous solubility profile of amorphous and crystalline (γ-form) indomethacin (Hancock and Parks, 2000). A fall in solubility of the amorphous form indicates precipitation of the stable polymorph.

It should be noted that with the amorphous state (as compared to the crystalline state), the solution reaches the super-saturation faster as compared to the crystalline state and hence the apparent solubility at any point before the saturation may be higher. The continuous removal of the dissolved solid from the saturated solution would avoid the process of recrystallisation from the solution. Inability to avoid the super-saturation may lead to precipitation of the more stable crystalline form and the equilibrium would reach when the entire amorphous solid transforms into the
crystalline solid. In this scenario the equilibrium solubility of the amorphous solid would be the equilibrium solubility of the crystalline solid (Figure 1-12). Hence the ability of the amorphous solid to reach the saturation faster is the true solubility advantage of the amorphous state over the crystalline counterpart.

Figure 1-13: Dissolution of amorphous and crystalline form of an anticancer drug (Albano et al., 2002).

The solubility advantage of amorphous state has been studied for many drugs. A comparative dissolution of the amorphous and the crystalline form of an anticancer drug is shown in Figure 1-13 (Albano et al., 2002). The dissolution profile shows 3-4 times improved solubility of the amorphous state over the crystalline state. There are several reports about the increased solubilization of the amorphous state e.g. 10 fold for novobiocin (Mullins and Macek, 1960), 2.5 fold for tetracycline (Miyazaki et al., 1975) and 1.4 fold for indomethacin (Imaizumi et al., 1980). The solubility advantage measured practically has been reported to be considerably lower than predicted using the measured thermal properties of a drug e.g. 25-104 fold for indomethacin, 112-1652 folds for glibenclamide etc. (Hancock and Parks, 2000). The authors also reported a difficulty in practically measuring the solubility of the amorphous state due to precipitation of the stable/metastable polymorph (Figure 1-12).
1.13 Bioavailability and the solubility advantage of amorphous state

The first and foremost aim of any formulation scientist is to achieve the required blood level of an active ingredient. Many drugs due to their low aqueous solubility can pose a significant challenge to obtain sufficient bioavailability via the oral route of delivery. The above mentioned methodology of use of the amorphous phase with higher apparent solubility can be translated into high bioavailability, provided that the absorption of drug is not limited by the permeability of drug through gut lumen. There is little published work about the bioavailability advantage of the amorphous phase over the crystalline phase. A comparative dissolution of the amorphous and the crystalline form of an anticancer drug suggested 3-4 times improved solubility of the amorphous state over the crystalline state. The improvement in solubility of the amorphous state is clearly seen in the bioavailability profile of this lipophilic drug (Table 1-5) when administered orally by capsules to dogs.

The solubility advantages of the amorphous drug do not always translate into the bioavailability improvements e.g. lisinopril (ACE inhibitor). The amorphous form has 15 fold solubility improvement as compared to the crystalline state of lisinopril (Roberts, 2003); although the bioavailability of the crystalline and the amorphous state of the drug as a melt dissolving tablet remained the same. The authors have not discussed the effect of tabletting on the amorphous state of the drug (since compaction and compression may induce recrystallisation) but they have concluded this to be due to low permeability of the drug through the gut lumen and hence poor bioavailability.

<table>
<thead>
<tr>
<th>Formulation</th>
<th>( \text{AUC}_{\infty}/\text{Dose} )</th>
<th>Tmax (h)</th>
<th>Cmax (ng/ml)</th>
<th>% Bioavailability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Micronized crystals</td>
<td>29.5±8.3</td>
<td>1.0±0.0</td>
<td>55±17</td>
<td>4</td>
</tr>
<tr>
<td>Nanosized crystals</td>
<td>86.1±13.7</td>
<td>1.5±0.6</td>
<td>142±53</td>
<td>11</td>
</tr>
<tr>
<td>Amorphous</td>
<td>468±87</td>
<td>3.4±1.9</td>
<td>874±452</td>
<td>61</td>
</tr>
<tr>
<td>IV formulation</td>
<td>766±82</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

Table 1-5: Pharmacokinetics of anticancer drug in dogs, oral dose 10mg/kg. Micronized and non-micronized drug administered as suspension, the amorphous form as a mixture with lactose in a capsule (Albano et al., 2002), (US patent 6492530).
<table>
<thead>
<tr>
<th>Class</th>
<th>Description</th>
<th>Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Class I</td>
<td>High solubility – high permeability</td>
<td>Paracetamol, Theophylline.</td>
</tr>
<tr>
<td>Class II</td>
<td>Low solubility – high permeability</td>
<td>Nifedipine, Ibuprofen, Griseofulvin, Ketoconazole, carbamazepine, Dapsone, Danazole, Troglitazone, Glibenclamide, Atovaquone, Furosemide, Rifampicin, Sulfamethoxazole, Trimethoprim</td>
</tr>
<tr>
<td>Class III</td>
<td>High solubility – low permeability</td>
<td>Acyclovir, Cimetidine.</td>
</tr>
<tr>
<td>Class IV</td>
<td>Low solubility low permeability</td>
<td>Chlorthiazide</td>
</tr>
</tbody>
</table>

Table 1-6: BCS and the examples of drugs coming under various classes (Lindenberg et al., 2004).

Understanding this scenario Class II drugs (Biopharmaceutic classification system) (Amidon et al., 1995) could be generalized to be the potential candidates for the use of the amorphous form as a means of bioavailability improvement. There are least chances that the bioavailability of a Class III and Class IV drugs would be improved by using the amorphous state. Perhaps, drugs at the borderline of Class II/IV (Albendazole), and Class I/II (Amitriptylline) etc. could also be the good candidates.
1.14 Summary

Although accompanied with many advantages, the amorphous state of actives may be the last choice to be used in solid dosage form. This is mainly due to its tendency to form a more stable crystalline state. The studies on amorphous state in pharmaceutical sector are mainly focused around,

A. The rate of crystallisation of amorphous state under different conditions.

B. To identify the conditions, which are safe for handling and storage of amorphous state

C. To stabilize the amorphous state of active using different approaches e.g. solid dispersions.

The identification of handling and storage conditions (temperature and humidity) is mainly based on glass transition temperature. The recrystallisation tendency of the amorphous form is linked to the molecular mobility. The prediction of the stability for the amorphous state is based on estimation of molecular mobility and a temperature below which there is no molecular mobility (Kauzmann temperature Tk). It has been generally observed that molecular mobility becomes insignificant at temperature about 50°C below Tg (Tg-50), so that the recrystallisation can be avoided over the shelf life of the product.

The amorphous state can uptake considerably more amount of water as compared to crystalline state. The amount of water uptake depends on the RH of the conditions to which the solid is exposed. The absorbed water can act as a plasticizer by lowering the Tg of dry state. The plasticisation effect of water further complicates the estimation of molecular mobility in the amorphous state making the prediction of Tk unrealistic. Most of the techniques used for characterisation of amorphous solids lack proper control over RH conditions and this can induce an error in the estimation of Tg. The techniques used to characterise amorphous state are no longer limited to Differential scanning calorimetry (DSC), Powder X-Ray diffraction and Dynamic mechanical analysis (DMA). Various spectroscopic techniques such as IR, NIR, NMR and Raman, Dielectric analysis, Thermally stimulated current (TSC) analysis are being employed to further understand the amorphous state.
1.16 Aim of the thesis work

Use of the amorphous active in solid dosage forms is mainly hampered due to its instability and tendency to transform into the crystalline form. The transformation is further accelerated beyond a certain range of temperature and RH conditions. Different substances in their amorphous form exhibit different crystallisation tendencies. The aim of this work was to identify the conditions of temperature and humidity under which the molecular mobility in the amorphous state ceases to an extent which leads to an insignificant crystallisation over the shelf life of a product. The major challenge facing this work was to identify analytical technique/s to study the amorphous state under a variety of conditions of temperature and humidity. It was also important to select the model glassy states which could be used along with the analytical techniques.

The characterisation of the amorphous state in a drug product such as a tablet could be attempted by first characterizing the amorphous state of drug as such and then in combination with other excipients or as a drug product.
1.17 Model substances used in the study

The amorphous states of following model substances have been used in the current study.

1.17.1 Indomethacin (B.P. 2002)

Chemical name | [1-(4-chlorobenzoyl)-5-methoxy-2-methylindol-3-yl]acetic acid
Formula | C_{19}H_{16}CINO_{4}
Formula weight | 357.8

Action and use | Anti-inflammatory; analgesic.
Preparations | Indomethacin Capsules, Indomethacin Suppositories

Characters

A white or yellow, crystalline powder, practically insoluble in water, sparingly soluble in alcohol.

Melting point | 158°C to 162°C.

Physical Properties

Indomethacin is known to exhibit three different crystal forms, a stable γ-form (m.p. 154°C, 101J/gm), a meta-stable α-form (m.p. 148°C, 87.6J/gm) and a benzene solvate β-form (Kaneniwa et al., 1985). The amorphous state of indomethacin has been widely studied by Zografi and co-workers, it exhibited a glass transition temperature of 47°C (Hancock et al., 1995). According to the studies on crystallisation kinetics of amorphous indomethacin it gets transformed into the stable γ-form or meta-stable α-form depending on the storage conditions (Andronis et al., 1997).
1.17.2 Nifedipine (B.P. 2002)

Chemical name  dimethyl 2,6-dimethyl-4-(2-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate
Formula  C\textsubscript{17}H\textsubscript{16}N\textsubscript{2}O\textsubscript{6}
Formula weight  346.3
Action and use  Calcium antagonist
Preparations  Nifedipine Capsules

Characters

A yellow, crystalline powder, practically insoluble in water, freely soluble in acetone, sparingly soluble in ethanol.

When exposed to daylight and to artificial light of certain wavelengths, it readily converts to a nitrophenylpyridine derivative. Exposure to ultraviolet light leads to the formation of a nitrophenylpyridine derivative.

Melting point  171°C to 175°C.

Physical properties

Nifedipine has been reported to exhibit three polymorphic modifications and four different solvates (Caira et al., 2003). The amorphous form of nifedipine exhibited a glass transition temperature of 48.6°C (Aso et al., 2000).
1.17.3 Lactose

Chemical name: Monohydrate of \(O\-b\-D\)-galactopyranosyl-(1\(\rightarrow\)4)-a-D-glucopyranose

Formula: \(C_{12}H_{22}O_{11},H_2O\)

Formula weight: 360.3

Action and use: Pharmaceutical aid.

Characters

A white or almost white, crystalline powder, freely but slowly soluble in water, practically insoluble in alcohol.

Physical properties

Lactose has been reportedly exhibited an anhydrate form and a monohydrate form.
The amorphous form of lactose exhibited a glass transition temperature of 113°C.

Storage

Store in an airtight container.
Chapter 2  Materials and Methods
2.0 Materials and Methods

This section of the thesis deals with various materials and methods used for generation of the amorphous state and various techniques used to characterise the amorphous state.

2.1 Materials

<table>
<thead>
<tr>
<th>Material</th>
<th>Supplier</th>
</tr>
</thead>
<tbody>
<tr>
<td>Indomethacin (γ-form)</td>
<td>Sigma</td>
</tr>
<tr>
<td>α-Lactose Monohydrate</td>
<td>Borculo Whey Products, (UK)</td>
</tr>
<tr>
<td>Nifedipine</td>
<td>Sigma</td>
</tr>
<tr>
<td>Liquid Nitrogen</td>
<td>BOC</td>
</tr>
<tr>
<td>Lithium Chloride, LiCl</td>
<td>Avocado Research Chemicals</td>
</tr>
<tr>
<td>Magnesium Bromide, MgBr₂</td>
<td>Aldrich</td>
</tr>
<tr>
<td>Sodium Dichromate, Na₂Cr₂O₇</td>
<td>Sigma</td>
</tr>
<tr>
<td>Sodium Iodide, NaI</td>
<td>Avocado Research Chemicals</td>
</tr>
<tr>
<td>Magnesium Nitrate, Mg(NO₃)₂</td>
<td>Avocado Research Chemicals</td>
</tr>
<tr>
<td>Sodium Bromide, NaBr</td>
<td>Avocado Research Chemicals</td>
</tr>
<tr>
<td>Copper Chloride, CuCl₂</td>
<td>Avocado Research Chemicals</td>
</tr>
<tr>
<td>Potassium Iodide, KI</td>
<td>Avocado Research Chemicals</td>
</tr>
<tr>
<td>Sodium Nitrate, NaNO₃</td>
<td>Avocado Research Chemicals</td>
</tr>
<tr>
<td>Sodium Chloride, NaCl</td>
<td>Avocado Research Chemicals</td>
</tr>
<tr>
<td>Potassium Chloride, KCl</td>
<td>Avocado Research Chemicals</td>
</tr>
<tr>
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<td>Avocado Research Chemicals</td>
</tr>
<tr>
<td>Potassium Sulphate, K₂SO₄</td>
<td>Avocado Research Chemicals</td>
</tr>
<tr>
<td>Methanol</td>
<td>BDH</td>
</tr>
<tr>
<td>Ethanol</td>
<td>BDH</td>
</tr>
<tr>
<td>n-Propanol</td>
<td>BDH</td>
</tr>
<tr>
<td>Dimethyl formamide</td>
<td>BDH</td>
</tr>
<tr>
<td>Hermetic and non-hermetic sealable aluminium pans</td>
<td>Perkin Elmer</td>
</tr>
</tbody>
</table>
2.2 Generation of amorphous solid state

The various methods which, could be used for generation of the amorphous state have been described briefly in section 1.4. In this work amorphous state was generated using quench-cooling of liquid melt (detailed in 1.4.1) for indomethacin and nifedipine whereas by spray drying (detailed in 1.4.2) was used for lactose from aqueous solution.

2.2.1 Preparation of amorphous indomethacin

About 4-5 gm crystalline indomethacin (γ-form), was placed in a porcelain furnace which was then heated over a wire gauge using a bunsen burner. The viscous, bright yellow and clear liquid indomethacin melt was then poured drop-wise into a flask containing liquid nitrogen which to obtain yellow glassy beads of amorphous indomethacin. Glassy indomethacin was then immediately transferred into an evacuated desiccator containing P₂O₅ to remove trapped moisture. The dry amorphous solid beads were ground lightly using a mortar and pestle and then passed gently through a 250# sieve (<350μ). The amorphous powder of indomethacin was again dried over P₂O₅ and stored at -70°C until it was used for further experiments. The amorphous solid prepared in this manner lasted for 2-3 months after which fresh amorphous solid was prepared. Every time the bottle containing amorphous solid was taken out it was equilibrated at room temperature to avoid moisture uptake from the atmosphere.

2.2.2 Preparation of amorphous lactose by spray drying of aqueous solution

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Setting value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inlet temperature (°C)</td>
<td>125</td>
</tr>
<tr>
<td>Outlet temperature (°C)</td>
<td>78</td>
</tr>
<tr>
<td>Feed rate (mL/min)</td>
<td>2</td>
</tr>
<tr>
<td>Pressure (bar)</td>
<td>3</td>
</tr>
<tr>
<td>Solution concentration (% w/v)</td>
<td>10</td>
</tr>
<tr>
<td>Atomizer air flow rate (norm liter/h)</td>
<td>400</td>
</tr>
</tbody>
</table>

Table 2-1: Spray drying parameters for the preparation of amorphous lactose.

About 50g of lactose monohydrate was dissolved in purified water (500 ml) to obtain a 10% w/v solution. The aqueous solution of lactose was then spray dried using a Buchi 190 mini spray dryer (Buchi, Switzerland). The technique was completely established for the production of the amorphous state of lactose (Chidavaenzi et al., 1997). The spray drying parameters used are given in Table 2-1. The amorphous
lactose was collected from the product collecting vessel and transferred to a desiccator containing \( \text{P}_2\text{O}_5 \), which was then dried under evacuation for 24h. The amorphous state of powder was confirmed from lack of sharp peaks in X-ray powder diffraction pattern.

The amorphous lactose prepared by the above mentioned method was used for measurement of water uptake by DVS, studying water induced transitions by TAM and estimating the glass transition using IGC.

2.2.3 Preparation of amorphous nifedipine

The amorphous nifedipine was prepared by two methods, by quench cooling of liquid melt and by spray drying of ethanolic solution.

2.2.3.1 Quench cooling of liquid melt

The amorphous state of nifedipine was generated by using the similar method to that described for the amorphous indomethacin, amorphous solids were used without grinding (2.2.1). The amorphous nature was confirmed by XRPD and DSC analysis and then was used for NIR studies.

2.2.3.2 Spray drying of ethanolic solution

Spray drying from the ethanolic solution of nifedipine was performed using SD MICRO™ spray dryer. (Niro A/S, Denmark) with Maxigas Nitrogen generator. Briefly, 2.9gm of nifedipine was dissolved in 170 ml of ethanol to which 20 ml of water was added. The ethanolic solution was then spray dried to using dry nitrogen stream as a carrier gas with with following optimized parameters.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atomizer gas flow</td>
<td>2.0 kg/h</td>
</tr>
<tr>
<td>Chamber inlet flow</td>
<td>20.0 kg/h</td>
</tr>
<tr>
<td>Inlet temperature</td>
<td>69.0°C</td>
</tr>
<tr>
<td>Chamber temperature</td>
<td>48.0°C</td>
</tr>
</tbody>
</table>

The solids were collected from the collection pot only and then dried under vacuum for 12h; the amorphous state was confirmed using DSC. The amorphous solids were stored at -20°C until further analysis. The amorphous nature was confirmed by XRPD and DSC analysis and then was used for DVS studies.
2.3 Preparation of 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 magnetic stirring facility. The saturated salt solution was then cooled down to ambient temperature and poured into a glass desiccator. The vapour pressure of pure water was lowered due to the dissolved salt. Each salt due to different saturation solubility lowered the vapour pressure of pure water to a different extent and hence produced a different RH atmosphere in the sealed desiccator. The RH value produced by each salt solution was reported by (Nyqvist, 1983) and is shown in Table 2-2.

<table>
<thead>
<tr>
<th>Salt</th>
<th>Temperature (°C)</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 Nitrate, NaNO₃</td>
<td>75.4</td>
</tr>
<tr>
<td>Sodium Chloride, NaCl</td>
<td>75.5</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 2-2: The relative humidity values of the saturated salt solution (Nyqvist, 1983).
2.4 Humidity treatment

About 50-55 mg of amorphous indometacin was weighed in a clear glass vial and placed into desiccator pre-equilibrated with salt solution at ambient temperature (taken as 25°C) for about 24h. Amorphous solid samples with sorbed water were then taken out and quickly sealed into hermetically sealed aluminium pans and then studied using Hyper-DSC as described in section 2.5.3.

Similar studies were performed using amorphous nifedipine (spray dried) but with amber coloured glass vials and 6h of equilibration time, after which the samples were studied using Hyper-DSC.
2.5 Differential scanning calorimetry (DSC)

DSC is used mainly to study transitions, chemical and physical, induced in a sample due to heating treatment. DSC is the modified version of differential thermal analysis (DTA) which is based on observing temperature difference between the sample and the reference pan (temperature of the sample remains constant until the change in sample is complete). DSC observes heat flow changes in a sample as it is heated. The instrumentation available is based on two principles; one is heat flux (single heating furnace) and second is power compensation (separate heating furnaces for sample and reference sides). For all the work done in this work Pyris-1 DSC (Perkin-Elmer) was used which operates on the power compensation principle.

![Diagram of DSC setup](image.png)

**Figure 2-1:** A schematic representation of power compensation DSC technique (Reproduced from Perkin-Elmer user manual).

The Pyris-1 DSC involves, two separate furnaces (for sample and reference pan) heated with separate heating resistors (Figure 2-1); two separate platinum sensors to detect the temperature of the sample and reference pans. The temperature detectors and the heaters interact in a way that allows the temperature of both sides to be kept the same (thermal null) at any time during the experiment; this is achieved by supplying differential power to each furnace. Data is presented in the form of differential heat supplied to the sample side in order to keep the temperature the same as the reference side. During any endothermic change, heat is taken into the sample and extra power is supplied to the sample side; whereas during an exothermic change heat is liberated from the sample and hence less power is supplied to the sample as compared to the reference side.

The sample is characterised using DSC with respect to temperature and heat of the thermal transition, e.g. melting temperature (Tm) and heat of fusion (ΔHf) associated with melting of the crystalline solid. DSC can also be used to measure specific heat capacity of the substance (C_p). The characterisation of the amorphous state using DSC is based on estimation of the glass transition temperature (Tg) observed as a jump in
specific heat capacity ($\Delta C_p$). In the pharmaceutical arena, DSC has been very useful in characterisation of polymorphs with respect to form purity and stability (Byrn et al., 1999).

Instead heating the sample in a normal temperature ramp it could also be heated with temperature modulations (i.e. alternate heating and cooling steps) using modulated temperature differential scanning calorimetry (MTDSC) (Craig and Royall, 1998). This heating programme can separate the overlapping thermal events such as relaxations (irreversible events) from glass transition (reversible event) in a single heating scan. The same information can also be obtained using a heating programme involving steps of heating and isothermal conditions (Figure 2-2) (Merzlyakov and Schick, 2001).

### 2.5.1 StepScan DSC analysis

![Temperature programme used in StepScan DSC, consisting repetitions of alternant steps of heating and isothermal treatment; along with the corresponding, untreated heat flow.](image)

**Figure 2-2:** Temperature programme used in StepScan DSC, consisting repetitions of alternant steps of heating and isothermal treatment; along with the corresponding, untreated heat flow.

The StepScan was undertaken using Pyris-1 DSC (Perkin-Elmer). About 5-7 mg of sample was taken in non-hermetically sealed aluminium pans; starting from 10°C sample was heated in steps of 1°C at the rate 2°C/min and then held isothermal at each step for 0.5 min, this was repeated 60 times, to get a scan from 10 to 70°C. Data obtained was processed by software provided by Perkin-Elmer. The glass transition response was recorded from the thermodynamic Cp line (as a reversible transition).
The enthalpy relaxation was calculated by integrating the part of IsoK baseline, corresponding to glass transition temperature range. Calibration of DSC for temperature and enthalpy was undertaken using indium as a standard at 2°C/min heating rate. Nitrogen was used as a purge gas at 20 ml/min.

2.5.2 Heating rate dependence of the glass transition

About 5-7 mg of amorphous indomethacin was taken into non-hermetically sealed pans and heated at various scanning rates (10, 50, 100 and 400°C) under a purge of nitrogen gas (20 ml/min.). DSC was calibrated for temperature and enthalpy at individual scanning rate using indium as a standard.

2.5.3 Hyper-DSC analysis

About 3-5 mg sample was placed in the aluminium pan and then scanned from -50 to 250°C at 300°C/min using the Pyris 1 (Perkin-Elmer), which was previously calibrated at same rate using Indium. Nitrogen was used as a purge gas at flow rate 20 ml/min. Studies involving the effect of sorbed water on the glass transition of the amorphous state were performed using hermetically sealed pans, for all other studies non-hermetically sealed pans were used.

Hyper-DSC analysis was used to characterise the amorphous solids prepared by various techniques also it was used to study the effect of sorbed water on the glass transition temperature of amorphous indomethacin and nifedipine. It was also used to characterise the amorphous solids after testing for DVS, TAM and IGC.

In the case of studies on plasticisation of amorphous state by sorbed water (section 3.3) Hyper-DSC analysis was performed in triplicate for each humidity point.
2.6 Isothermal microcalorimeter

Whereas the sample is scanned over a range of temperature using DSC, the isothermal microcalorimetry involves recording heat flow from the sample by holding it at constant temperature.

2.6.1 Principle

Almost all changes in the universe are accompanied with heat exchange. Exothermic change takes place with the release of heat, whereas endothermic change is associated with intake of heat. These changes are defined on the basis of two relative terms, the system where actual process or processes take place and the surrounding in which the system is in thermodynamic equilibrium. Exothermic processes are accompanied with a rise in temperature of the system whereas, endothermic processes with a drop in temperature. If the surrounding is maintained at an isothermal temperature, any change in temperature of the system will lead to a temperature gradient between the system and the surrounding. Isothermal microcalorimetry is based on heat flow or heat leakage principle where, the heat produced in a thermally defined vessel flows away in an effort to establish the thermal equilibrium with its surroundings; isothermal calorimetry measures the heat exchanged (Figure 2-3). Applications of this technique have been reviewed for pharmaceutical development (Buckton et al., 1999; Buckton and Darcy, 1999).

Figure 2-3: The channelling and detection of heat flow through thermopile blankets (Peltier element) in TAM (Reproduced from TAM Manual).
2.6.2 Instrumentation

In all the studies performed in this work a Thermal activity Monitor TAM (2277, Thermometric AB, Järfalla, Sweden) was used. Thermal stability was achieved by utilizing a 25 litre water thermostat which surrounded the reaction/measuring vessel and acted as an infinite heat sink (Figure 2-4) (Thermometric AB, 1985).

![Diagram](image)

**Figure 2-4:** Outline of the functional design of TAM.

Reactions could be studied within 5-80°C, the working temperature range of thermostat. Up-to four individual measuring vessels could be housed in the water thermostat, where they are maintained at a temperature constant to within ±10°C; this allowed fractions of microwatts to be measured accurately. Each measuring cylinder was sufficiently isolated from others to assure that the measurements in each cylinder were independent of each other. This facility could allow four different measurements or reactions to be studied at a time. Heat exchange (between sample and sink) was channelled through extremely sensitive thermopile blankets (Figure 2-3), the Peltier elements. Peltier elements along with a metal heat sink formed the main heat measuring device or measuring cup and could respond to temperature gradients of less than one millionth of a degree Celsius. The actual heat measurement was based on the 'twin measuring principle'. This meant that each measuring cylinder constitutes two measuring cups (Figure 2-4). In the first cup, the sample containing ampoule was positioned, whereas in the second, the reference ampoule was
lowered. In each measuring cup, two Peltier elements were connected in series but in opposition so that the resultant signal represented a difference in heat flow from the two measuring cups. These detectors convert heat energy into a voltage signal proportional to heat flow and results were presented as a measure of thermal energy exchanged by the sample per unit time. Quantification of the results was performed by using electrical calibration where a known power value (or current) was passed through the built-in precision resistor. The calibration resistor was integral with measuring cup to simulate as near as possible the position of reaction. This ensured that output from the detector would be as near as possible, identical when the same power is dissipated from the resistor as from the sample.

**Figure 2-5:** Twin measuring principle of TAM for the detection of heat flow.

The amplifier was connected to each channel covering the measurement range to be selected between 3-3000μW. Amplification was done by sending the signal from measuring cup through an amplifier to the computer where, data were collected and presented via Digitam 4.1 for Windows® user interface (operating software).
2.6.3 Experimental

The different set-ups used included closed system (batch isothermal microcalorimetry) and open system (isothermal RH perfusion microcalorimetry).

2.6.3.1 Batch Isothermal Microcalorimetry

For all the studies 3 ml glass ampoules were employed for both sample side and the reference side. In each case the reference ampoule was set up to match the sample ampoule as closely as possible. About 30-40 mg of sample in the glass ampoule was exposed to the vapour pressure of mixture of solvents, ethanol: propanol (1:1) by placing a small tube containing those solvents into the glass ampoule. Same amount of solvent containing tube was placed into the reference ampoule. Each ampoule was then plugged using Teflon bung and then sealed with an aluminium cap. The ampoule was then hung on a lifter with the aid of a lifting loop and then lowered to the equilibrium position of into the TAM which, was previously equilibrated to the experimental temperature. Ampoules were lowered to the measuring position after 10 minutes of equilibration. Heat flow was recorded right from the equilibration position. All the experiments were carried out at 25°C.

2.6.3.2 Isothermal RH Perfusion Microcalorimetry

Relative vapour pressure in the sealed ampoule of the batch microcalorimetry could not be varied. This limitation could be overcome with a 2250 Micro Reaction RH perfusion insertion assembly (Thermometric AB, Järfalla, Sweden) (Figure 2-6). The RH perfusion assembly was primarily designed to control the vapour pressure of water (%RH) but could also be used without any modification with many organic solvents preferably alcohols. The perfusion assembly was lowered in the TAM channel which, allowed the measurement of the heat flow as the vapour pressure of the solvent was varied.

The relative humidity in the 4 ml steel ampoule was regulated with the flow of dry Nitrogen gas. The mainstream of the gas flow was divided into two streams, each passing through the mass flow controller (Bronkhorst Hi-Tech, Netherlands) individually. One stream of the gas (Figure 2-6) flows along the central shaft of the perfusion unit and opens directly into the steel ampoule (4ml) which, could be described as the dry flow. The other stream of the gas was passed through two humidifying chambers and saturated with vapours of the solvent stored into the chamber, finally opening into the steel ampoule. The ratio and the flow rates of the gases flowing through each stream could be varied using mass flow controllers to
produce the required %RH in the sample steel ampoule. Normally flow rates of 5-200 ml/min could be achieved with the flow controllers. Condensation of the solvent vapours in the gas was avoided by maintaining the outlet temperature slightly higher than the ambient temperature. This was done by using a heater at the outlet of the perfusion unit. The perfusion unit could be controlled using the Digitam software with the flow switch module installed in the 2280 TAM accessory interface (Thermometric AB, Järfalla, Sweden).

**Figure 2-6:** The schematic of Vapour perfusion unit by Thermometric (Sweden).

For all the experiments the flow rate of the dry gas was set to 100 ml/min using mass flow controllers. Humidifying wells were filled with 500µl of the solvent. Dry sample was weighed (30-40mg) in the steel ampoule and sealed using the perfusion unit. Experiments were designed as described in TAM Technical Note 012 (Thermometric AB, 2000a). The empty steel ampoule was used on the reference side.

In the step ramp %RH was increased in steps and kept constant before going to the next step. During the continuous ramp experiments, %RH was increased continuously at the fixed rate (3%RH/h). Before initiating the humidity ramp, the
sample was dried under the flow of dry gas which, was followed by observing the heat flow response. Continuous ramp experiments were performed at different temperatures (25, 35 and 40°C). For this purpose the water bath temperature and the system settings for the Thermostat were changed appropriately. %RH temperature corrections were done automatically as explained in the TAM Technical Note 013a (Thermometric AB, 2000b). The same assembly was used with different alcohols (methanol, ethanol and n-propanol) instead of water in the humidifying wells to control the solvent vapour pressure in the steel ampoule.

### 2.6.4 Calibration for the heat flow

Each channel was calibrated when the amplifier settings were changed or when the TAM was switched off. Calibration for batch experiments was performed separately using a solvent tube in the empty ampoule at both sides of the calorimeter channel (sample and reference side). Calibration for the perfusion experiment was performed as a part of the complete experiment, during the post drying stage and before the actuation of the ramp. Two glass ampoules (batch calorimetry) or two steel ampoules (perfusion experiments) were lowered to the measuring position of the TAM calorimeter channel. After equilibration (steady baseline) the signal was adjusted using the Zero dial to get the baseline signal at 0.000±0.100 µW. At this point an exact amount of heat was generated using a specific current through the channel resistor (described in section 2.6.2). The heat input was recorded as an exothermic response by Digitam which then settled at a particular heat flow value. This response was adjusted to the correct value (±0.100 µW) by means of fine adjusting dial. Once the signal was adjusted to the required value, the current through the resistor was switched off allowing the signal to return to the baseline. The signal was adjusted again to 0.000±0.100 µW using the Zeroing dial.

### 2.6.5 Validation of RH perfusion mass flow controllers

The validation of mass flow controllers is required in order to check accuracy of the RH generated in the steel sample ampoule of the perfusion unit (Buckton, 2000). The validation was performed by placing a small tube containing a saturated salt solution in the steel ampoule. The two humidifying chambers were filled with purified water (500 µl each). The gas flow was switched 'off' completely; the perfusion unit was then lowered to the measuring position and the heat flow was set to the 'zero' position. The RH was then set to a value reported as an 'equilibrium RH' generated, for the salt solution (e.g. 75.3% if saturated NaCl was used), after which the gas flow was switched 'on'. The heat flow was settled to an endothermic or exothermic value depending on the RH generated by the carrier gas over the salt solution in a steel ampoule. The RH value was then increased or decreased in a stepwise manner (1%
each step) until the heat flow crossed the zero value. The heat flow would settle to a value at 'zero' only if the RH of the gas flow was equal to the equilibrium RH generated for the particular salt solution.

For each validation experiment performed using NaCl saturated salt solution, the heat flow was settled to 'zero' at 75±1%RH, indicating the proper functioning of the mass flow controllers.
2.7 Solution calorimetry

When a substance (A) is added to substance (B) some heat is either released or absorbed depending on the nature of the interaction between the two substances. When substance A is solid and B is some solvent (e.g. water) in which substance A is soluble, then the heat released or absorbed is called as the 'enthalpy of solution', whereas in the case of dispersion processes (solid dispersed into liquid) the heat exchanged is called as the 'enthalpy of dispersion'.

The process of solubilization of solids could be viewed as breaking of intermolecular bonds holding the lattice in a solid and formation of the new bonds between solvent and solid molecules. Since different polymorphs are associated with different lattice energy they would possess different enthalpy of solution. The enthalpy of solution measured carefully could be very useful to estimate disordered parts (amorphous regions) in the crystal lattice (Fikal et al., 1978). The Solution Calorimetry has been demonstrated to be useful in estimation of very low levels of amorphous regions in the predominantly crystalline state (Hogan and Buckton, 2000).

2.7.1 Instrumentation and technique

Thermometric 2225 Precision Solution Calorimeter (Thermometric AB, Järfalla, Sweden) was used for all solution calorimetry experiments. The solution calorimeter was used with a very high stability (±0.0001°C) water bath of the Thermal Activity Monitor (TAM, Thermometric 2227). The main calorimeter constitutes three primary components, a calorimeter unit Figure 2-7, a calorimeter cylinder in which the unit was mounted during the experiment and a solution calorimeter module to link the calorimeter to the computer.

The calorimeter unit consists of a reaction vessel (100 ml, Pyrex glass) and a motor driven stirrer unit. In the glass vessel, a thermistor and a heater were permanently mounted descending from the top of the vessel. A sapphire breaking tip was mounted on a pin at the bottom of the vessel. The stirrer system consisted of a motor and a gold stirrer (which also function as a holder for glass ampoule). The stirrer system holding the sample ampoule was inserted into the reaction vessel and was mounted on a spring to function as a plunger. The entire system was pushed down at a point during the experiment where an ampoule was to be broken. The ampoule breaking led to release of its contents into the solvent and the heat was exchanged. The nature of interaction between the solvent and ampoule contents governed the heat exchange and ultimately led to the rise or fall in the temperature of the vessel. The temperature offset was measured by a thermistor and could be transformed into heat of the
reaction by using the electric calibration (passing known amount of current through the heater and measuring the temperature offset).

![Diagram of solution calorimeter unit]

**Figure 2-7:** The solution calorimeter unit by Thermometric.

The entire system could be operated between 0-90°C that means the measurements could be performed at different temperatures. The data collection was performed using the 'Software for Solution Calorimeter System' version 1.2.

### 2.7.2 Experimental

About 130-160 mg of the dry sample was weighed accurately in a glass ampoule, which was then plugged with a silicone bung and sealed with molten bees wax. The sealed ampoule was then loaded into the stirrer unit of the calorimeter which was then inserted into the glass vessel containing 100 ml of the solvent. The combined unit was then lowered into the solution calorimeter channel of TAM to the equilibration position. The stirrer speed was adjusted to 600 rpm. The heater was switched 'on' to heat the contents of the glass vessel to approximately 50-60 mK higher than the experimental temperature and then switched 'off'. The temperature was then allowed to settle by following the standard deviation (SD) in the temperature values. The ampoule break experiment was started when the SD was under 10μK.

The first part of the experiment consisted of electrical calibration during which 10J were introduced over the duration of 20 sec. The second part consisted the actual breaking of the ampoule by lowering the stirrer unit by a plunger which led to the
release of the ampoule contents into the solvent followed by dissolution. The third part consisted of a second electrical calibration with the same parameters as the first calibration. The temperature offset was constantly recorded at each event including two calibration events and the ampoule breaking event. The heat of solution was calculated using the 'Software for Solution Calorimeter System' version 1.2.
2.8 Vapour sorption analysis

This is a gravimetric technique and constituted monitoring the change in sample weight by exposing it to controlled relative humidity. It is based on the fact that when the sample is exposed to a relative humidity (%RH) condition it would absorb or desorb water vapours which would be directly reflected in the change in weight of the sample. Initially, the water sorption studies were undertaken by exposing the sample to a range of %RH conditions generated using salt solutions and recording the equilibrium water uptake. These studies were used primarily to determine the hygroscopicity of the sample (Kuroda and Nakagaki, 1963) and detecting the least hygroscopic salt. A sorption-desorption isotherm can give useful information of the sample about the surface area (from the BET plot) and the enthalpy of sorption process. Dynamic Vapour Sorption (DVS) has been adapted to study the weight change continuously in order to obtain the information which otherwise would be lost. Recently vapour sorption has been coupled with near infra red spectroscopy (NIR) to study the physical state transformations in amorphous solids (Lane and Buckton, 2000).

2.8.1 The DVS Technique

The vapour sorption studies done in this work utilized the Dynamic Vapour Sorption (DVS) apparatus by Surface Measurement Systems (SMS, London, UK). A schematic of the instrument is shown in Figure 2-8. It consisted of identical sample and reference pans (made up of quartz glass), hanging on two sides of the Cahn microbalance (Limit of detection ±1x10^-4mg). The whole assembly was housed inside glassware within which the %RH could be controlled. An accurate control over the %RH was achieved by using two mass flow controllers (MFC). The flow rate and the proportion of dry and moist gas were controlled by the MFCs. The entire system (microbalance and the glassware) was kept in an incubator where accurate temperature control could be achieved. Humidity and temperature probes were positioned close to the sample and the reference pans to give an independent reading of the system performance. The exact control over the experimental conditions (temperature and the %RH) could be achieved via a computer using the ‘DVS-1’ software (SMS, UK), which was also used for the data collection.

2.8.2 Experimental

About 40-50mg of sample was loaded into the sample pan, whereas an empty pan was used as a reference. Before loading the sample the temperature of the incubator was adjusted to the required value. A typical DVS experiment consisted of three parts. In
the first part sample was dried under 0%RH (dry gas flow) for about 3-4h. In the second part sample was exposed to increased humidity conditions for an extended period of time and in the third part humidity was decreased back to 0%RH. The weight change of the sample was followed over the entire experiment. Increasing the humidity in the second step was done in several ways as described below.

**Figure 2-8:** A schematic of Dynamic vapour sorption (DVS) apparatus.

### 2.8.2.1 Continuous ramp

After the initial drying period humidity was increased gradually and continuously at a fixed rate (3%RH/h) from 0 to 90%RH. These experiments were performed at various temperatures for amorphous indomethacin whilst at 25°C for amorphous lactose.

### 2.8.2.2 Stepwise ramp

After the initial drying period humidity was increased in the steps of 5 or 10%RH. The change in a RH step was either performed at the end of a fixed period or when the weight change of a sample reached an equilibrium value (from dm/dt criteria). The experiments were performed at various isothermal temperatures. Considering the duration of the experiment (2-3 days) and the availability of the DVS only the experiments at 25°C were studied to confirm the reproducibility whilst the experiments at higher temperatures were performed only once.
2.9 Inverse phase gas chromatography (IGC)

This is a reverse of the gas chromatographic technique. IGC is used to characterise the physicochemical properties of solids, mainly surface properties. Normal gas chromatography involves a standard column in which a mixture of gases to be separated is eluted using a carrier gas; whereas inverse gas chromatography involves solid under study to be packed in the form of a column and only one probe gas (with known physicochemical properties) at infinite or very low concentration being eluted at a fixed flow rate of carrier gas. The retention time of probe gas depends on its interaction with the solid surface. A series of IGC experiments with a wide range of gaseous probes could be used to measure the physicochemical properties of solid in detail. IGC has been demonstrated to be useful in estimation of surface energies, acid/base properties, solubility parameters, sorption isotherms and phase transition.

2.9.1 Instrumentation of IGC

![Diagram of IGC Instrumentation](Figure 2-9: An overview design of inverse gas chromatograph (IGC) by Surface Measurement System (SMS, UK).

For all IGC studies in this work a commercially available inverse gas chromatograph (iGC) by surface measurement system (SMS, UK) was used. This is a modified version of Agilent 6890 GC gas chromatograph. The overview design of the iGC is shown in Figure 2-9. It consists of a flow control system to control the flow rate of gases, a solvent oven chamber in which all solvent probes are stored along with humidifying bottles, a column oven chamber to control temperature of the column, a valve system to control the flow path carrier gas and detectors viz. thermal conductivity detector (TCD) and flame ionization detector (FID). TCD mainly detects the humidity level of
the eluting gas and comes first (hence called back detector) followed by FID (front
detector) which detects all the gaseous probes by burning them.

**Figure 2-10:** Schematics of iGC by SMS

**Figure 2-11:** IGC valve position showing the gas flow pathway during the standby
mode (A) and during the injection mode (B).

The instrumentation design allows two separate gas flows—sample and reference. Sample flow carries the organic probe gases whereas the reference flow is devoid of any probe gases. Both the flows could be humidified using a separate water bottle for each flow (Figure 2-10). This assembly can assist in studying the retention behaviour under controlled humidity conditions. The position of the valves selects the flow path of the carrier gas. The location of four different valves is shown in Figure 2-11. Valve 1 selects the flow of gas through either of the two columns (left or right). Valve 2 rotates the carousel through 9 different solvent bottles to make a choice between nine
different probe injections. Valve 3 injects the loop contents and valve 4 selects gas
flow to the column (reference and sample). The valves are actuated using compressed air (pneumatics) and are controlled using the computer. The flow path of the carrier gas is maintained by opening and closing the various valves during the stages of the IGC experimentation as shown in Figure 2-11.

The flow control system consists of six mass flow controllers (MFCs) to regulate the flow rate of the gases (mainly carrier gas), humidity level of the carrier gas and concentration of the probe gas to be injected. The position of each flow controller is shown in Figure 2-10.

2.9.2 Experimental

The experimental work involved mainly two things, preparation of column packing and conditioning, then performing the elution method.

2.9.1.1 Column preparation and conditioning

About 300-400 mg of amorphous solid was packed in pre-silanated glass columns (internal diameter 3 mm) using a standardized tapping method, which involved tapping the filled column for a fixed duration (10 min.) at fixed intensity. The packed column was then placed in a column oven (temperature stable over ±0.2°C of the set value) of gas chromatograph and conditioned at 30°C, using dry He (purity >99.996%, moisture level <6ppm) (BOC, UK) as a carrier gas at a flow rate of 20 sccm (standard cubic centimetres per minute) for 4h at 0%RH to remove any adsorbed or absorbed water and other gasses from the packed sample. RH, which was initially set at 0%, was then sequentially increased in steps. Conditioning at each RH step was performed for 40 min. before performing the injections of probe gases. Moisture levels in the carrier gas were detected using the thermal conductivity detector (TCD). Control of RH was through mass flow controllers which were electronically calibrated by Surface Measurement Systems and are designed to give the same level of RH control that is obtained for DVS experiment.

2.9.1.2 The elution method

Elutions using decane (Acros Organics, purity >99%) injections with concentrations 0.08%p/p° were performed at a flow rate of 5 sccm of He at the required %RH, methane (BOC, UK) was used as internal standard. The solvent oven temperature was maintained at 50°C (±0.2°C). Elution times of decane and methane were measured using flame ionisation detector. Retention volumes Vmax and Vcom were calculated using IGC analysis software (Version 1.2 standard) from the time for maximum peak height and centre of mass of the retention peak respectively. The
pressure drop across the column was monitored at each RH/T using pressure transducers fitted to the IGC.

The elution experiments were performed in two ways, in one way the humidity was held constant at a particular %RH value and the temperature was increased (after each temperature increment elution of decane was performed) whilst in another way the temperature was held constant and humidity was increased. Each experiment was repeated at least three times. These experiments were performed in order to study the transitions in amorphous indomethacin and amorphous lactose.

2.10 Near infrared spectroscopy (NIR)

Near infrared spectroscopy was performed using a specially adapted optical reflectance NIR probe (Foss NIRSystems, Maryland, USA). The NIR probe was the part of modified DVS apparatus adapted to record the NIR spectrum simultaneously with the weight change (Figure 2-8). The NIR probe was positioned approximately 4mm under the quartz, flat bottomed sample pan of the DVS instrument. The collection of the NIR spectra was programmed using Vision® software (Version 2.2). The each spectrum was recorded as log 1/R as a function of wavelength, where R is the reflectance.
Chapter 3  Differential scanning calorimetry to study amorphous state
3.0 Differential scanning calorimetry to characterise amorphous state

Differential scanning calorimetry (DSC) is being used routinely to characterise the solid state e.g. estimation of melting temperature or glass transition temperature. Different ways of heating the samples have been identified to better characterise the physical state of the solid. Conventionally, a slow heating rate of about 10-20°C/min is used but now the advantages of heating at a faster rate up to 500°C/min (Hyper-DSC) have been identified. Heating at fast rates can lower the chances of temperature induced phase transitions in the sample (e.g. polymorphic inter-conversion or crystallisation of amorphous solid). Hyper-DSC has been proved especially important in magnifying the glass transition response (Saunders et al., 2004). Since the power output is a function of time, the step change response observed using the high heating for glass transition will be larger. A heating scan with intermittent isothermal steps (StepScan DSC) can be used to separate the glass transition from the overlaying relaxation peak. The StepScan differs from Modulated DSC in that an isothermal step is used (in StepScan) instead of a cooling step (in MDSC).

This section describes characterisation of the amorphous state using differential scanning calorimetry (DSC). The effect of heating rate (10-400°C/min) on glass transition temperature (Tg) for the amorphous state was studied. The activation energy (for molecular relaxation) and fragility index of the glass were calculated using heating rate dependence of Tg. The effect of sorbed water on the glass transition was studied for the amorphous state of indometacin and nifedipine using high speed DSC (300°C/min). Finally StepScan DSC was used to separate the glass transition and relaxation response. Enthalpy relaxation measurements performed were then used to estimate the molecular mobility for different glasses of indometacin, lactose and nifedipine.
3.1 Calorimetric transitions in amorphous solids

The amorphous state shows typical transitions when heated in a DSC as exemplified for amorphous indomethacin (Figure 3-1). Amorphous indomethacin undergoes a glass transition ($T_g$) accompanied with a small endothermic peak due to structural relaxation, an exothermic peak ($T_c$) due to possible recrystallisation followed by further endothermic melting transitions ($T_{m1}$, $T_{m2}$) for the re-crystallised forms.

![DSC scan](image)

**Figure 3-1:** DSC scan for amorphous indomethacin at 10°C/min heating rate showing the transitions typical of an amorphous state.

The glass transition is a temperature at which the amorphous state converts between highly viscous glassy to a less viscous rubbery state. Above $T_g$ molecular mobility in the amorphous state increases which induces crystallisation at $T_c$. Although $T_g$ is a characteristic of an individual glass it depends on the way it is measured and the presence of sorbed water.
3.2 Estimation of activation energy by heating rate dependence of Tg

The dependence of Tg on heating rate could be seen from Figure 3-2 for amorphous indomethacin. It was clear that with a higher scan rate the glass transition shifted to a higher temperate (Tg shifted from 48.2°C at 10°C/min to 61.5°C at 400°C/min), although the melting was observed at almost constant temperature. The DSC furnace was calibrated for each heating rate using indium as a standard. The height of the specific heat change (ΔCp) at the glass transition was also increased, although accurate estimation of ΔCp at individual heating rate is difficult due to the overlapping relaxation response.

**Figure 3-2:** Heating rate dependence of glass transition temperature for amorphous indomethacin, 2-3mg dry sample placed in non-hermetically sealed pans. * glass transition, ** crystallisation, *** melting.

Heating rate dependence of the glass transition has been reported for several aqueous glasses (Her and Nail, 1994) and was used to estimate the activation energy for the relaxation process accompanying glass transition using equation 19 (Moynihan et al., 1974).

\[
\frac{d \ln |q|}{d \left( \sqrt{T_g} \right)} = - \frac{\Delta h^*}{R}
\]

Equation 19
where, \( q \) is heating rate (K/min), \( T_g \) glass transition temperature, \( \Delta h^* \) is activation energy of the relaxation process and \( R \) is universal gas constant (\( R=8.1345 \) J/mole/K). \( \Delta h^* \) calculated from equation 19 was used to calculate the fragility parameter \((m)\) from equation 20.

\[
m = \frac{\Delta h^*}{2.303RT_g}
\]  

Equation 20

The plot of inverse temperature against \( \ln(\text{heating rate}) \) is shown in Figure 3-3. The slope of this line was used to calculate the activation energy for the relaxation process at the glass transition. This value was also shown to be similar to the activation energy for the viscous flow (Moynihan et al., 1974). Also shown in Figure 3-3 is the comparison with reported values for the heating rate dependence of \( T_g \) for amorphous indomethacin. The reported studies cover the heating rate range from 5-40K/min whereas in this work a broad heating range from 10-400K/min was covered.

![Figure 3-3](image)

**Figure 3-3:** The heating rate dependence of the \( T_g \) for amorphous indomethacin, data compared with reported values Reference 1 (Ramos et al., 2004) and Reference -2 (Hancock et al., 1998b).

The slope of the lines were very different ultimately affecting activation energy (244.6 kJ/mole in this work compared to the reported value of 385kJ/mole). There could be several possible reasons for the above mentioned difference in activation energy values \((\Delta h^*)\) observed in different studies for amorphous indomethacin. The most logical
reason could be the amount of absorbed water in the amorphous solid. Although all the studies were performed with dried amorphous solid, its exposure to the environmental RH could not be avoided which can influence the measured Tg value and hence activation energy values ($\Delta h^*$). Since the calculation of the fragility index is based on $\Delta h^*$ value (equation 20) difference in $\Delta h^*$ value could be potentially carried over and may lead to erroneous estimation of the fragility index value ($m$).

![Figure 3-4](image-url)

**Figure 3-4:** The behaviour of glasses with different fragility indices in the supercooled liquid region, reproduced from (Bohmer et al., 1993).

The concept of strong against fragile glass formers was explained in the Introduction (1.6.1). A higher value of the fragility index indicates a more fragile or weaker glassy solid. It could be seen from Figure 3-4 that the log $t$ value (relaxation time) for strong glasses ($m=16$) increases almost linearly as the temperature is reduced towards Tg (Tg/T=1). Whereas in the case of fragile glasses ($m=200$) log $t$ value decreases exponentially with a more pronounced change near Tg. Analogous to the relaxation time other mechanical properties (e.g. viscosity) follow an exponential change near Tg for a fragile glass former. Although the properties of the glass former in the supercooled region are used to classify the glasses it is anticipated that the trend in properties of the glass (e.g. relaxation time and viscosity) would follow the similar relationship below Tg (solid state of the glass). This means that the strong glass would continue a linear decrease in the properties whereas the fragile glass would follow a sudden change near Tg. The properties of the fragile glass would change more drastically as the Tg is approached during the heating or cooling of a glass former. A fragile glass is expected to show a faster crystallisation around the glass transition region as compared to further below Tg. The implication of strong and fragile glass classification criteria is important during the estimation of the zero mobility
temperature (Tk); a criterion which could be used in the pharmaceutical arena to avoid the crystallisation over the shelf life of the amorphous product. An accurate estimation of activation energy ($\Delta h^*$) and fragility index ($m$) could be of great use in comparing the differences in the crystallisation behaviour of different glasses.

<table>
<thead>
<tr>
<th></th>
<th>$\Delta H$ (kJ/mole)</th>
<th>$T_g$ (K)</th>
<th>$m$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nifedipine</td>
<td>351</td>
<td>320</td>
<td>58.63</td>
</tr>
<tr>
<td>Phenobarbital</td>
<td>343</td>
<td>316</td>
<td>57.96</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>385</td>
<td>319</td>
<td>64</td>
</tr>
<tr>
<td></td>
<td>244.7$^b$</td>
<td></td>
<td>40.7$^b$</td>
</tr>
<tr>
<td>$B_2O_3$</td>
<td>384</td>
<td>554</td>
<td>32</td>
</tr>
</tbody>
</table>

Table 3-1: The Activation energy and the fragility index values ($m$) for different glasses from, $^a$(Aso et al., 2001), $^b$(Bohmer et al., 1993) and $^c$ this work.

The fragility of a glass is independent of glass transition temperature ($T_g$) but depends on the activation energy of the glass transition. A comparison of $m$ values for organic and inorganic glasses is shown in Table 3-1. $B_2O_3$ is an inorganic glass ($m$=32) comparatively stronger than other organic glasses.

The presence of moisture in amorphous solid could profoundly affect the estimation of activation energy and $m$ value. In the case of citric acid glass activation energy decreased from 733 kJ/mole in the dry state, to 410 kJ/mole with 8.6% w/w of water. This was accompanied with corresponding decrease in $m$ value from 135 to 86 upon hydration (Lu and Zografi, 1997). The hydration of a glassy solid could lead to variation in observed $m$ values among the different reports as the DSC measurements lack the control over the humidity conditions.
3.3 Plasticisation of amorphous state by sorbed water

The effect of sorbed water on glass transition temperature of the amorphous state has been studied by using conventional DSC (slow heating rate 10K/min.) and by modulated DSC (Royall et al., 1999). The use of hermetically sealed pans during the heating scan can avoid evaporation of the sorbed water whereas the fast heating rate minimizes its redistribution in the amorphous solid. The use of a hermetically sealed pan along with high scanning speed could therefore be the best way to study the effect of sorbed water on Tg of amorphous solid.

3.3.1 Plasticisation of amorphous indomethacin by water vapour

![Graph](image)

**Figure 3-5:** DSC scan for amorphous indomethacin using a hermetically sealed pan at 300°C/min, A. dry solid, B. stored at 11.3%RH showing two Tg s one below 50° (circle) along with the second at around 60°C.

For dry amorphous indomethacin the glass transition could be seen at around 60°C as a step change in the specific heat (Figure 3-5A). The glass transition was followed by a small endothermic peak (c.a. 90°C) which could be due to collapse or the structural relaxation of the amorphous state. A relaxation response is generally observed as a part of the glass transition response (Figure 3-1), the delay (two separate transitions, Tg at 60°C and relaxation at 90°C) observed could be associated with the use of hermetically sealed pan. A pressure build up during the heating scan could prohibit the structural collapse of amorphous state immediately after glass transition. The absence of melting transitions (around 150°C) along with the absence of recrystallisation (around >100°C) could altogether mean that indomethacin was predominantly in the amorphous state and the fast heating rate could prohibit the nucleation and the recrystallisation.
Figure 3-6: DSC scans (300°C/min) using hermetically sealed pan, for amorphous indomethacin stored at different humidity conditions for 24h at 30°C. The glass transition was clearly shifted to a lower temperature with increasing humidity, c.a. 60°C at 11.3%RH to c.a. 30°C at 97.3 %RH.

The plasticisation effect of absorbed water on amorphous indomethacin was shown in Figure 3-6 and Table 3-2. The glass transition was gradually shifted to a lower temperature as %RH was increased. The gradual decrease was due to the increasing levels of the sorbed water which acts as a plasticizer.

With the progressive plasticisation, the width of the glass transition broadened (width $\Delta T_g = T_g \text{ onset} - T_g \text{ end}$) indicating spreading of the glass transition over a broader temperature range. The broadening of the $T_g$ was also accompanied by a fall in $\Delta C_p$ (step change in the specific heat) at the glass transition. The broadening of glass transition due to absorbed water is illustrated in Figure 3-7A and Table 3-2 ($\Delta T_g$). Amorphous indomethacin stored at 97%RH showed considerably higher $\Delta T_g$ (15.8°C) than the sample stored at 11.3%RH (11.4°C). The broadening of width of glass transition has often been correlated to increased distribution of molecular relaxation times (different amorphous domains relaxing differently) and could lead to a change in fragility of the amorphous state (Hancock et al., 1998b).
Figure 3-7: The Effect of sorbed water on glass transition response of amorphous indomethacin from DSC scan (300°C/min) using hermetically sealed pans, A. Comparison between glass transition for storage at 11.3 and 97.3%RH. B. Comparison between first and second heating scan after storage at 97.3%RH, the width of glass transition was reduced during the second scan.

<table>
<thead>
<tr>
<th>%RH</th>
<th>Tg onset (°C)</th>
<th>ΔTg (°C)</th>
<th>ΔCp (J/g/K)</th>
</tr>
</thead>
<tbody>
<tr>
<td>11.3</td>
<td>60.6 ± 0.7</td>
<td>11.4±0.5</td>
<td>0.67±0.07</td>
</tr>
<tr>
<td>30.7</td>
<td>52.3 ± 0.4</td>
<td>10.6±0.2</td>
<td>0.76±0.04</td>
</tr>
<tr>
<td>38.2</td>
<td>51.9 ± 0.2</td>
<td>8.7±0.2</td>
<td>0.75±0.16</td>
</tr>
<tr>
<td>57.5</td>
<td>44.5 ± 0.4</td>
<td>10.9±0.7</td>
<td>0.66±0.07</td>
</tr>
<tr>
<td>74.3</td>
<td>43.3 ± 0.9</td>
<td>11.4±0.3</td>
<td>0.50±0.04</td>
</tr>
<tr>
<td>75.3</td>
<td>38.4 ± 0.3</td>
<td>10.8±0.9</td>
<td>0.50±0.09</td>
</tr>
<tr>
<td>80.9</td>
<td>37.1 ± 0.8</td>
<td>16.8±2.6</td>
<td>0.40±0.04</td>
</tr>
<tr>
<td>84.3</td>
<td>35.2 ± 0.6</td>
<td>12.3±2.2</td>
<td>0.50±0.05</td>
</tr>
<tr>
<td>93.7</td>
<td>30.1 ± 0.9</td>
<td>14.3±2.2</td>
<td>0.44±0.06</td>
</tr>
<tr>
<td>97.3</td>
<td>26.1 ± 0.5</td>
<td>15.8±2.6</td>
<td>0.49±0.16</td>
</tr>
</tbody>
</table>

Table 3-2: A list of onset temperature for the glass transition (Tg onset), width of the glass transition ΔTg and specific heat change ΔCp for amorphous indomethacin after the storage at various RH conditions at 30°C.

The broadening of glass transition could also imply inability of water molecules to distribute uniformly in the amorphous solid (considering the hydrophobic nature of indomethacin). This could mean that if plasticised solid was reheated it may show decrease in ΔTg value during the second scan (as compared with the first heating scan) due to the redistribution of the water during the first heating scan. Indeed this was the case with amorphous indomethacin. The reduction in width of glass transition in the second run (ΔTg=13.0°C) could easily be observed as compared to the first scan (ΔTg=20.0) (Figure 3-7B) with the onset of Tg remaining the same.
The uneven water distribution in the amorphous solid could lead to different regions being plasticised to different extent. Under extreme situations of uneven water distribution multiple glass transitions could be observed during DSC scans. Multiple glass transitions were not detected regularly, may be due to a technical limitation of the instrument or a heating rate of 300°C/min may not be high enough to avoid the redistribution of sorbed water in the solids. A multiple glass transition was however clearly observed for solids stored at 11.3%RH (Figure 3-5B), under this storage condition the sorbed water could be just adsorbed at the surface of the solid.

Although the glass transition shifted to a lower temperature, the endothermic peak (which was correlated to relaxation) observed c.a. 90°C did not shift until 80.9%RH (Figure 3-6). After 80.9%RH the peak was very small for 84.3%RH and at 97.3%RH it was completely absent (Figure 3-6 and Figure 3-7B). It was highly likely that the endothermic peak could be due to relaxation or collapse in the amorphous state. Disappearance of the peak (after 84%RH) could mean that amorphous indomethacin existed in a relaxed state (or in a rubbery state). This could mean that the amount of sorbed water at ≥84% RH lowered the dry Tg to or below the experimental temperature. Hence under these conditions of the temperature and RH amorphous indomethacin could exist in a rubbery state.

![Graph](image.png)

**Figure 3-8:** The Tg of amorphous indomethacin containing absorbed water (Tg mix) measured using DSC (300°C/min) plotted against water content measured by DVS.

The endothermic melting transition at 0°C was absent for all amorphous solids containing different amounts of water. This could mean that water molecules existed
as a molecular dispersion with amorphous indomethacin, as the existence of separate water phase would have resulted in an ice melting transition at 0°C. Water, when present in high amounts may exist as a separate phase (similar to condensed water) and in this state water is highly mobile and can have a catalysing effect on many solid state reactions. The state of water is particularly important when water contents are high, such as 3 %w/w of dry amorphous indomethacin when stored at ≥90%RH.

The values of Tg (onset) measured after storage under different RH conditions, as a function of amount of sorbed water (measured using the DVS) are shown in Table 3-2. Initially a small amount of absorbed water had a considerable plasticizing effect on amorphous indomethacin; about 0.6%w/w of absorbed water lowered Tg of dry solid by about 16°C. The plasticizing effect then levelled until about 1.5% w/w water; Tg was then reduced gradually dropping by about 18°C as a consequence of the next 1.4% w/w of absorbed water. Overall about 2.96% w/w of water reduced Tg of the dry solid from 60.6°C to 26.1°C (by 34.5°C). This was in comparison with the previous studies using slow heating rate (1°C/min), where Tg of amorphous indomethacin was lowered from 43°C (in dry conditions) to 10°C (with 2.8%w/w of water), a total drop by 33°C (Andronis et al., 1997). The plasticisation effect of water was observed to be much higher than that predicted using the Gordon Taylor equation (Figure 3-8). This was observed in previous studies for amorphous indomethacin (Andronis et al., 1997). The Gordon Taylor equation (equation 16) is based on the perfect volume additivity for the components of a mixture. Lowering of Tg for amorphous indomethacin is due to an increase in total free volume of indomethacin molecules in the amorphous state. Deviation of actual Tg values from the Gordon Taylor prediction was reported for hydrophobic polymers like PMMA (Hancock and Zografi, 1994) and was supposed to be due to various polar and non-polar interactions between polymer and water molecules.
3.3.2 Plasticisation of amorphous nifedipine by water vapour

Figure 3-9: DSC heating scans (300°C/min) for amorphous nifedipine (spray dried) stored at various RH conditions for 6h at 30°C; glass transition (c.a. 60°C) clearly disappearing beyond 52.8 %RH.

The plasticisation effect of absorbed water observed by exposing amorphous nifedipine to various RH conditions was found to be much more complex than the effect of absorbed water on the amorphous indomethacin. The heating scans after exposure of amorphous nifedipine to various RH conditions are shown in Figure 3-9 and Figure 3-10. Amorphous nifedipine exhibited a clear glass transition typical of an amorphous state for storage at 0%RH. The melting transition along with the exothermic crystallisation observed in all the scans could be due to two possibilities; one the presence of partially crystalline spray dried amorphous state or second the heating rate of 300°C/min was unable to prevent crystallisation. The glass transition for amorphous nifedipine stored at 0, 11.3 and 38.3%RH was distinct and consistent. But for storage at ≥52.8%RH the glass transition response started diminishing. Occurrence of multiple transitions with wavering baseline was obvious from DSC scans for amorphous nifedipine stored at ≥52.8%RH (Figure 3-10).

Results of calorimetric glass transition temperatures (Tg onset and ΔTg) corresponding to the biggest step change in specific heat capacity after exposure to various RH conditions are shown in Table 3-3.
Table 3-3: Temperature at which the calorimetric glass transition response was observed for amorphous nifedipine after storage at various RH and 30°C, along with the change in the specific heat capacity obtained from DSC scans from Figure 3-9.

Exposure of amorphous nifedipine to 0-75.3%RH did not affect the glass transition temperature but at higher RH Tg decrease from 52.2°C at 75%RH to 43.7°C at 97.3%RH. High values of the ΔCp obtained for the sample exposed to the 0 and 11.3%RH (Table 3-3) could be due to the high scanning rate together with the enthalpic relaxation process which accompanies the glass transition response.

Exposure to >38.2%RH resulted in fading of the calorimetric glass transition response, this was observed as a drop in the ΔCp value from 0.86 J/g/K at 0%RH to 0.25 J/g/K at 97.3%RH. A drop in ΔCp values could be due to splitting of a single glass transition response into multiple transitions as seen from wavering of the baseline (Figure 3-10). Multiple glass transitions could be due to differential distribution of sorbed water in amorphous nifedipine and hence differential plasticisation. Due to differential lowering of the glass transition, some regions of amorphous nifedipine may exist in the relaxed state (since the relaxation is faster near the glass transition temperature). Another possibility for drop in ΔCp values could be the strengthening of the amorphous glassy state as a result of the sorbed water. A decrease in fragility value was reported for citric acid due to sorbed water (Lu and Zografi, 1997) and the strengthening of a glass could be associated with a fall in ΔCp values at the glass transition (Angell, 1996). The third possibility could be a partial transformation of amorphous state into the crystalline state and thereby reducing ΔCp at glass transition.

DSC scans for samples exposed to ≥52%RH showed several transitions beyond the Tg region, which could be anticipated considering the number of polymorphs and solvate structures exhibited by nifedipine. Three polymorphs primarily reported in are form I (m.p. 174°C) form II (m.p. 163°C) and form III (m.p. c.a. 135°C) (Keymolen et al., 2003) as well as solvates and the hydrate forms (Caira et al., 2003).
Figure 3-10: DSC heating scans (300°C/min) for amorphous nifedipine (spray dried) stored at various RH conditions for 24h at 30°C; individual heating scans from Figure 3-9. Red and black lines indicate multiple DSC analysis.

Although the possibility of transformation of amorphous nifedipine into one of the polymorphic or the hydrate structures during the exposure to the high RH could not
be ignored, it was not of interest for the current work to characterise the number of polymorphs or hydrates generated due to exposure to high RH.
3.3.3 Comparison between the plasticisation of amorphous indometacin and nifedipine by water

In the case of amorphous indometacin the plasticisation effect of water was gradual, i.e. Tg was lowered gradually with the increasing RH or amount of absorbed water. Whereas for amorphous nifedipine Tg was not affected much until 52-75%RH, after which there was a sudden drop.

Another difference observed between the two amorphous states was the step change in heat capacity at glass transition (ΔCp). For amorphous nifedipine, ΔCp decreased sharply by about 0.5J/gm/K with the exposure to low RH such as 38.2%RH (Table 3-3). Whereas in the case of amorphous indometacin ΔCp changed by 0.1-0.2J/gm/K over the entire range of RH studied (0-97%RH) (Table 3-2).

Generally Tg is used as a criteria to assess the stability of the glass. This means a glass with higher Tg should be more stable than a glass with lower Tg. It follows that two glasses with an equal Tg should have a similar stability profile. Although two glasses used in the current study exhibited a similar dry Tg (60°C for indometacin and 57°C for nifedipine) their crystallisation tendencies were very different.

The changes observed for amorphous nifedipine due to water vapour exposure could be intermediate to the process of crystallisation. The amorphous state of nifedipine could be more prone to crystallisation in humid conditions as compared to amorphous indometacin. It is possible to conclude that the changes in molecular mobility are higher for amorphous nifedipine compared to amorphous indometacin under humid conditions.
3.4 Estimation of molecular mobility in amorphous state

In this section estimation of molecular mobility in the amorphous state of indomethacin, nifedipine and lactose at various temperatures below $T_g$ are considered. The empirical Kohlrausch-Williams-Watts equation (equation 4) has been used along with enthalpy relaxation measured by StepScan DSC to calculate average relaxation time. Since the kinetics of the crystallisation process are well understood in the amorphous state of indomethacin, nifedipine and lactose, it would be possible to correlate the crystallisation process to molecular mobility. It should also be possible to estimate temperature conditions for low molecular mobility so as to avoid the crystallisation process.

3.4.1 Estimation of enthalpy relaxation using StepScan DSC

![Graph showing heat flow against temperature with labels for glass transition and enthalpy relaxation]

**Figure 3-11:** The differentiation of total heat flow into reversible (Thermodynamic $C_p$ line) and non-reversible (IsoK baseline) heat flows using StepScan DSC for amorphous indomethacin (heating programme detailed in the methods section).

<table>
<thead>
<tr>
<th></th>
<th>$T_g$ $\pm$ $C_p$ Extr ($^\circ$C)</th>
<th>$\Delta C_p$ (J/g/$^\circ$C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Indomethacin</td>
<td>46.59 $\pm$ 0.09</td>
<td>0.39 $\pm$ 0.01</td>
</tr>
<tr>
<td>Nifedipine</td>
<td>45.85 $\pm$ 0.04</td>
<td>0.37 $\pm$ 0.01</td>
</tr>
<tr>
<td>Lactose</td>
<td>115.25 $\pm$ 0.56</td>
<td>0.56 $\pm$ 0.04</td>
</tr>
</tbody>
</table>

**Table 3-4:** The parameters of glass transition obtained by using StepScan DSC.

The total heat flow obtained using the StepScan heating programme was separated into reversible (Thermodynamic $C_p$ line) and non-reversible (IsoK baseline) heat flows using StepScan software of Pyris-1 DSC (Sichina, 2000). The results obtained for a
glassy state of indomethacin are shown in Figure 3-11. The glass transition being the reversible response was observed along Thermodynamic Cp line as a step change in specific heat and enthalpic recovery processes being non-reversible observed along IsoK baseline as an endothermic peak. The characteristic parameters of glass transition estimated from Thermodynamic Cp line for indomethacin, nifedipine and lactose are shown in Table 3-4. The area of the endothermic peak observed along the IsoK baseline was calculated to give an estimate of enthalpy of the relaxation process. A similar response for enthalpic recovery and glass transition was also observed with the amorphous state of nifedipine and lactose using exactly similar StepScan heating programmes. Generally, conventional DSC was used to study the relaxation process in the amorphous state (Hancock et al., 1995) although, using the conventional DSC the two processes, the glass transition and the recovery are observed on top of each other making their individual estimation difficult. The modulated DSC was reported to be able to study the glass transition and the enthalpy recovery responses individually (Craig et al., 2000).

3.4.2 Estimation of aging from enthalpic recovery

![Graph showing the effect of aging at 30°C on the enthalpic recovery for amorphous nifedipine, the peak height increased with the extended aging as did the peak area.]

**Figure 3-12:** The effect of aging at 30°C on the enthalpic recovery for amorphous nifedipine, the peak height increased with the extended aging as did the peak area.
Figure 3-13: The effect of aging at 30°C (A) and 35°C (B) on the enthalpic recovery for amorphous indomethacin, peak height increased with extended aging.

Figure 3-14: The effect of aging at 80°C on enthalpy recovery of amorphous lactose.

The aging studies were performed by holding amorphous material isothermally for various intervals and then performing StepScan DSC analysis. The endothermic peak responses obtained for aging of amorphous nifedipine at 30°C are shown in Figure 3-12. The peak height and ultimately the peak area increased with increasing the aging duration. It was also clear from Figure 3-12 that the peak max was shifted to a higher temperature with increased aging duration. Since the peak corresponds to the enthalpy recovery process, the peak max corresponds to the temperature at which the maximum numbers of molecules undergo relaxation recovery. The peak shift to a higher temperature could be correlated to consolidation of the glass with increased aging. Amorphous indomethacin (Figure 3-13) and amorphous lactose followed similar
trends in enthalpy recovery and temperature shift for the peak max. The aging studies were followed over an extended duration of time for amorphous lactose, about 8-17 days as compared to 24h for indometacin and nifedipine.

In the case of amorphous lactose shift in the peak max temperature was also accompanied by breakdown of single glass transition response into multiple transitions immediately one after another, this could be seen as a discontinuity in step change response (Figure 3-15). We are not aware of this having been reported previously.

**Figure 3-15:** The effect of aging at 80°C on glass transition response for amorphous lactose observed along specific heat line using StepScan DSC. Arrows indicate the discontinuity in glass transition response.

**Figure 3-16:** The enthalpy relaxation as a function of time obtained for amorphous lactose at different aging temperatures.
The enthalpy relaxation values obtained by aging studies for amorphous lactose increased with longer aging time (Figure 3-16). At Tg-15°C (100°C) an initial increase in enthalpy relaxation was followed by a plateau that could be observed clearly between 8-24 h (Figure 3-16). A similar plateau effect was also observed for aging at Tg-25°C. The enthalpy relaxation values at Tg-35 (70°C) followed a gradual increase even after 8 days. Amorphous nifedipine and indometacin followed similar trends in enthalpy relaxation values.
3.4.3 Estimation of molecular mobility

Enthalpy relaxation values obtained by aging the amorphous state were used to calculate the extent of relaxation as explained in the section 1.9.1 using equation 5 and 6. The extent of relaxation for lactose at different temperatures were plotted as a function of aging time (Figure 3-17) and fitted in equation 4 using $\tau$ and $\beta$ as variables. Fitting the extent of relaxation at each temperature in the empirical KWW equation produced values of $\tau$ and $\beta$. The values of average relaxation time ($\tau$) obtained for lactose, indomethacin and nifedipine are shown in Table 3-5 and Table 3-6. For all compounds used in this study $\tau$ values increased as aging temperature was lowered below Tg.

<table>
<thead>
<tr>
<th>Temperature</th>
<th>$\tau$ (h)</th>
<th>$\beta$</th>
</tr>
</thead>
<tbody>
<tr>
<td>60°C (Tg-55)</td>
<td>8.9 x 10^{-1} ± 6.45 x 10^{-4}</td>
<td>0.03 ± 0.27</td>
</tr>
<tr>
<td>70°C (Tg-45)</td>
<td>30526.8 ± 175292.4</td>
<td>0.3 ± 0.24</td>
</tr>
<tr>
<td>80°C (Tg-35)</td>
<td>606.3 ± 275.4</td>
<td>0.6 ± 0.12</td>
</tr>
<tr>
<td>90°C (Tg-25)</td>
<td>76.1 ± 15.2</td>
<td>0.4 ± 0.04</td>
</tr>
<tr>
<td>100°C (Tg-15)</td>
<td>6.2 ± 1.07</td>
<td>0.4 ± 0.06</td>
</tr>
</tbody>
</table>

Table 3-5: Average relaxation time ($\tau$) and distribution parameter ($\beta$) obtained at different aging temperatures for amorphous lactose.

![Figure 3-17](image.png)

Figure 3-17: The extent of relaxation as a function of time for amorphous lactose; solid lines are the curves of the best fit in the empirical KWW equation (equation 4).
Table 3-6: Average relaxation time (τ) and distribution parameter (β) obtained at different aging temperatures for amorphous indomethacin and nifedipine.

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Indomethacin (Tg = 46.6°C)</th>
<th>Nifedipine (Tg = 45.9°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>τ (h)</td>
<td>β</td>
</tr>
<tr>
<td>35°C</td>
<td>16.93 ± 2.4</td>
<td>0.29 ± 0.02</td>
</tr>
<tr>
<td>30°C</td>
<td>17.58 ± 1.1</td>
<td>0.7 ± 0.04</td>
</tr>
<tr>
<td>25°C</td>
<td>34.13 ± 3.1</td>
<td>0.98 ± 0.1</td>
</tr>
<tr>
<td>15°C</td>
<td>1.6*10^8 ± 10^8</td>
<td>0.19 ± 0.1</td>
</tr>
</tbody>
</table>

In the case of amorphous lactose (Table 3-5) τ values increased from about 6h at Tg-15°C to about 600h at Tg-35. For amorphous indomethacin reducing the temperature from Tg-10 to Tg-20 increased the τ values from 17h to 34h as compared to an increase from 1h to 3000h over the same temperature range for amorphous nifedipine. This is interesting considering the similar Tg of both amorphous compounds (Table 3-4).

At lower aging temperature extremely long relaxation times (τ) with large error values were observed e.g. Tg-45°C for lactose, Tg-30 for amorphous indomethacin and nifedipine (Table 3-5 and Table 3-6). Large error values were also reported for amorphous sodium indomethacin (Tong and Zografi, 1999) and for amorphous lactose (Craig et al., 2000). This could mean that the existing model was unable to predict extremely long relaxation time.

Figure 3-18: A comparison of relaxation times obtained for amorphous state of nifedipine, indomethacin and lactose below Tg.
The inability to predict extremely long relaxation time at a particular aging temperature could be regarded as relative\(^1\) absence of molecular mobility. Under this scenario the temperature below which the molecular mobility disappears could be regarded as the zero mobility temperature (Tk, Kauzmann temperature). Alternatively the large error in \(\tau\) values could mean the insensitivity of the existing method to detect extremely slow molecular motions. Enthalpy recovery values obtained over 12 days were insufficient to predict the average relaxation time for amorphous lactose at \(T_g\)-45°C (using the empirical KWW equation). The detection of slow relaxation processes would need aging of the amorphous state to be studied over several months or years.

The average molecular relaxation time has often been used to predict shelf life stability of the amorphous state. In an approach to predict the shelf life of the amorphous state, the distribution of relaxation times has been used instead of average molecular relaxation time (Shamblin et al., 2000). In the present work molecular relaxation time has been used only for the comparison of the molecular mobility in the amorphous state below \(T_g\) and not to predict the shelf life. Since the purpose of the current study was to compare the molecular mobility in different glasses the prediction of the shelf life was not attempted.

The direct comparison of molecular relaxation times at different aging temperatures for three glasses was performed in Figure 3-18. Different temperature scaling methods have been proposed for the purpose of comparison between different glasses. These methods involve A) the ratio of aging temperature to glass transition temperature (\(T/T_g\)) and B) the difference between glass transition temperature and aging temperature (\(T_g-T\)). In this work \(T_g-T\) was used as a temperature scaling method. This means log of relaxation time was plotted against \(T_g-T\) for different glassy states (Figure 3-18). It can be seen from Figure 3-18 that different glasses follow similar aging temperature dependence until 20°C below \(T_g\) (\(T_g-T= 20\)). A further decrease in aging temperature (beyond \(T_g-T=20\)) resulted in different behaviour for three glasses. It could be possible to relate the different behaviour of three glasses to the fragility index and hence to crystallisation tendency.

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\(^1\) Relative to higher aging temperature, e.g. aging at \(T_g-15°C\) for amorphous lactose.
3.5 Summary of characterising amorphous state by DSC

In this chapter the characterisation of the amorphous state using DSC was studied. The results obtained could be summarized as follows,

- Dependence of Tg on heating rate was studied for amorphous indomethacin. The variation in Tg was used to calculate the fragility index for the amorphous state. The use of fragility index values in comparing the stability of different glasses has been elaborated. Since the absorbed water can affect Tg of the amorphous state and ultimately the estimation of fragility index it is necessary to control the water content of the sample and the RH conditions of measurement.

- The plasticisation effect of absorbed water was demonstrated for the amorphous state of indomethacin and nifedipine using hyper DSC. Whereas absorbed water lowered Tg gradually and continually for amorphous indomethacin, the effect was different for amorphous nifedipine. ΔCp lowering effect of absorbed water was more pronounced in the case of amorphous nifedipine as compared to indomethacin. At higher RH it was difficult to visualize the glass transition from ΔCp for amorphous nifedipine. In the case of amorphous indomethacin the plasticisation effect deviated from the Gordon Taylor prediction. The observed deviation could be due to uneven water distribution in the amorphous solid. Hyper-DSC was used successfully to study many attributes of the plasticizing effect of absorbed water for amorphous solids e.g. broadening of ΔTg, lowering of ΔCp, multiple glass transitions and collapse of the glassy state (from absence of a relaxation event for amorphous indomethacin at 93.7%RH).

- StepScan DSC was used to separate the glass transition response from enthalpy recovery. This enabled the aging study of the amorphous state at different temperatures below Tg. The molecular mobility was estimated using the empirical KWW equation for three glasses of indomethacin, nifedipine and lactose. A comparison of molecular mobility among these glasses could be performed in order to study their crystallizing tendency. The lowering of aging temperature below Tg was accompanied with increase in average relaxation time (hence decrease in molecular mobility) for all three glasses. Further lowering of aging temperature resulted in extremely long relaxation times, which could not be measured using the current model. This temperature could be near to the zero mobility temperature (Tk), where molecular mobility is absent in amorphous solids. The relaxation process and hence molecular mobility could be successfully studied using StepScan DSC coupled with the empirical KWW equation.
Chapter 4 Gravimetric studies on the interaction of water vapour with amorphous solid
4.0 Gravimetric studies on the interaction of water vapour with amorphous solid

The amorphous state reportedly interacts very differently with water vapour as compared to the crystalline state, allowing relatively more free access to the ingress of the water vapour (Ahlneck and Zografi, 1990) which is then held in the amorphous state as a sorbed water. The sorbed water can influence many physical and chemical properties of the amorphous solid.

The amorphous states of different substances have different interactions with water vapour, which further depends on the physicochemical properties of the amorphous form e.g. glassy or rubbery nature. In this chapter of the interaction of water vapour with amorphous state of lactose, indometacin and nifedipine at different isothermal conditions is reported.
4.1 Interaction of water vapour with amorphous lactose

The water vapour sorption for the amorphous lactose was studied by exposing the amorphous solid to a stepwise increment in RH, whilst continuously recording the weight change as described in methods (section 2.8.2.2).

4.1.1 Water sorption isotherm of amorphous lactose

![Water vapour sorption isotherm for amorphous lactose 25°C; shape of the isotherm very similar to Type II. Dotted circle shows the shoulder and arrows show inflection points on the isotherm.](image)

The water vapour sorption behaviour of amorphous lactose at 25°C is shown in Figure 4-1. The amount of water vapour uptake into the amorphous solid increased with the increase in RH; at 55%RH about 12% water was absorbed for the dry weight of the solids. A sigmoidal isotherm with was observed in the 0-55%RH range. Above 55%RH the spontaneous loss of absorbed water was observed. It has been reported that amorphous lactose absorbed >11% of the water when exposed to 75%RH, followed by spontaneous desorption due to crystallisation of amorphous lactose (Lane and Buckton, 2000). Such a high amount of water sorption is not unusual for amorphous solids e.g. amorphous PVP K-90 absorbed about 60% water (Oksanen and Zografi, 1990). Although the shape of the water sorption isotherm resembles type II BET-isotherm (Brunauer et al., 1940) its physical meaning could be misleading. A type-II BET\(^2\) isotherm has an initial linear adsorption followed by an inflection due to monolayer coverage of the gas molecules and then steep curvature due to multi-

\(^2\) A type II BET isotherm could be exemplified by adsorption of nitrogen on iron catalyst at -195°C.
layered adsorption or condensation. Although the modified BET equation takes\(^3\) into account the different adsorption processes viz. initial monolayer followed by multilayer adsorption and then condensation it fails to account for the changes in the physical nature of the adsorbent. It is now widely established that the absorbed water lowers \(T_g\) and increases molecular mobility (Oksanen and Zografi, 1990). The plasticisation effect of absorbed water means that at a certain level of absorbed water \((W_g)\) dry \(T_g\) would be lowered to the experimental temperature; after which the amorphous, glassy state with high viscosity would transform into a less viscous rubbery state. The rubbery state could exhibit different water uptake characteristics as compared to the glassy state. The BET equation does not account for any change in the physical nature of the adsorbent.

The following points on the isotherm were estimated to study the interaction of water with the lactose.

### 4.1.1.1 Critical point: 1; the onset of molecular mobility

The first critical point on the water sorption isotherm corresponding to monolayer coverage of water molecules was calculated using the BET equation (equation 11). It could be assumed that the initial linear part of the water sorption isotherm (before the shoulder) was due to adsorption of water, without significant effect on the physical state of amorphous lactose. The linear part fitted to BET equation (equation 11) is shown in Figure 4-2.

<table>
<thead>
<tr>
<th>(W_m)</th>
<th>(R_H_m)</th>
<th>(T_{g_m})</th>
<th>(T_{g_m} - T)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.5% w/w</td>
<td>17.2%</td>
<td>74.3°C</td>
<td>49.3</td>
</tr>
<tr>
<td>(*2.5% w/w)</td>
<td>(*16.0%)</td>
<td>(*83.0°C)</td>
<td>(*58.0)</td>
</tr>
</tbody>
</table>

**Table 4-1:** BET parameters calculated using the linear part of water sorption isotherm (0-25%RH) for amorphous lactose at 25°C; \(W_m\) - amount of water for monolayer coverage, \(R_H_m\) - Relative humidity corresponding to \(W_m\), \(T_{g_m}\) - glass transition temperature of binary mixture containing amorphous lactose and sorbed water, \(T\) - experimental temperature. \(^a\) literature values (Lechuga-Ballesteros et al., 2003), \(^b\) measured using DSC.

\(^3\) The modified BET equation is used to explain sigmoidal shape of the isotherm
Figure 4-2: BET plot for water sorption behaviour of amorphous lactose at 25°C.

The monolayer volume coverage calculated using the y-intercept of the linear plot equated to 2.5% w/w of water. Tg of the binary system containing 2.5% w/w of sorbed water in amorphous lactose was estimated to be 75.1°C, which is c.a. 50.1°C higher than the experimental temperature (T=25°C). The molecular mobility studies performed for dry amorphous lactose indicated undetectable molecular mobility at Tg-45 to Tg-55 (Section 3.4.3). It could be fairly argued that the binary mixture of amorphous lactose and water lacked molecular mobility below 2.5% w/w of water content at 25°C. Any amount of water sorbed, beyond 2.5% lead to increase in molecular mobility. Since water contents of 2.5% correspond to monolayer volume coverage (which is in the region of shoulder on water sorption isotherm); it is likely that monolayer coverage and shoulder on the isotherm correspond to the onset of molecular mobility in the amorphous binary mixture. The values of water content and RH which induce molecular mobility in amorphous lactose are shown in Table 4-1. The values estimated in this work match with the reported values.

---

4 Tg of the binary mixture containing amorphous lactose with absorbed water could be estimated using Gordon Taylor equation (Equation 15-17).

5 Since Tg of the binary system with amorphous lactose and 2.5% water is >50°C higher than the experimental temperature 25°C.
4.1.1.2 Critical point: 2; water induced glass transition of amorphous lactose

Figure 4-3: Mass change differential amorphous lactose at 25°C along with water sorption isotherm. Arrow shows the amount of sorbed water required to lower dry Tg to experimental temperature (25°C) (calculated by Gordon Taylor Equation).

The transitions induced in amorphous lactose due to water sorption could be seen more distinctly by plotting the differential of water sorption isotherm. In Figure 4-3, the differential change in mass for each step increase in RH is plotted and compared with the water sorption isotherm at 25°C. The differential weight change was calculated by measuring additional water absorbed during each 5% step rise in RH. It could be seen from Figure 4-3 that at each step change in RH between 25-40%RH, the sample sorbed more water than the previous step and a maximum was reached at 40%RH. From the differential water sorption behaviour there could be two critical points on water sorption isotherm; first at 25%RH where there was a rise in water sorption and second at 40%RH where differential sorption reached a maximum. If the rise in differential water uptake at 25%RH could be linked to any particular process (e.g. molecular rearrangements), it is very likely that the processes which were induced in amorphous lactose at 25%RH were approaching the completion at 40%RH. The RH required causing sufficient water sorption to lower dry Tg to the experimental temperature 25°C, (calculated by Gordon Taylor equation) equated to about 37%RH.

Part of water sorption isotherm before spontaneous crystallisation, which resulted in desorption of water.
Since beyond 40%RH there was a drop in differential water sorption over 45-55%RH, which is different from onset of crystallisation initiated at 60%RH (Figure 4-3).
Figure 4-4: A method described to estimate RH, which induces a glass transition in amorphous lactose at 25°C. The isotherm was obtained by ramping RH at the rate of 6%RH/h (from Burnett et al., 2004).

In a recent study on amorphous lactose a sorption isotherm was obtained by continuously ramping RH (instead of a stepwise RH ramp) (Figure 4-4) (Burnett et al., 2004). The curved region on the isotherm between 15-45%RH was related to a change in water uptake phenomena by amorphous lactose from adsorption to absorption and the point of intersection of tangents drawn to curved regions was claimed as a point of glass transition (Figure 4-4). It is possible that the RH value corresponding to the point of intersection of two tangents may also correspond to the glass transition event but it is always arguable whether or not the process of water uptake changes from adsorption to absorption in this region.

4.1.1.3 Critical point: 3; the collapse in amorphous lactose

The RH value at which the differential water uptake (Figure 4-3) was first time lower than the previous RH step (50%RH) could be correlated to collapse in the structure of amorphous lactose. Since the collapse follows glass transition it was evident that amorphous lactose existed in the rubbery state at 50%RH.
4.1.2 Water sorption kinetics of amorphous lactose

![Graph showing water sorption kinetics of amorphous lactose](image)

**Figure 4-5**: Dynamic vapour sorption trace for amorphous lactose with stepwise increments in the RH at 25°C.

The weight change in amorphous lactose recorded dynamically at each step of RH at 25°C is shown in Figure 4-5. The water sorption behaviour could be divided into three RH regions. The first RH region, up to 20%RH with small water uptake but rapid equilibration; the second RH region of small water uptake without equilibration (25 to 35%RH) and the last region of high water uptake with rapid equilibration (40 to 45%RH). A comparison of water sorption behaviour of amorphous lactose at individual RH steps is shown in Figure 4-6. Each trace in Figure 4-6 is a water sorption response for a 5%RH increase for the stated RH value (e.g. 15% is the step from 10-15%RH). The lines for 15 and 20%RH fall exactly on top of each other; such behaviour may be expected when water is adsorbed on the powder surface at.

The line for 25%RH shows slight deviation from the 20%RH line on extended exposure (after 100 min.). This means at 25%RH amorphous lactose is showing very slow but gradual water uptake without equilibration after 4 h. The line for 30%RH shows more rapid water uptake than 25%RH but without equilibration. At 35%RH water uptake was more rapid as compared to 30%RH but the equilibration did not occur in 4h. The water uptake at 40 and 45%RH was more rapid than the previous RH steps and the equilibrium was reached within 2 h.

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8 Since RH was increased in same amount each time (i.e. 5% step), a same amount of weight gain would follow until the process of adsorption is uniform.
Assuming that the water uptake process continues with formation of hydrogen bonds between water and lactose molecules and/or water and water molecules, the availability of binding sites on lactose accessible to water molecules would decide the rate and extent of water uptake. The initial small water uptake with equilibration (at 15 and 20%RH) could mean that bonding sites at the surface are getting covered. The slow water uptake without equilibration (at 25, 30 and 35%RH) could mean additional bonding sites are being created with the progressing water uptake. The increase in rate of water uptake at 30, 35 and 40%RH could mean that water molecules are penetrating into the bulk of the powder and creating channels for the further diffusion of water molecules to the core of the particle. The rate and extent of water uptake was much higher at 40 and 45%RH than at any other RH step indicating free diffusivity of water molecules throughout the bulk of the powder. The free diffusivity of water molecules could be linked with reduced viscosity of amorphous solid or the powder has transformed from highly viscous, glassy state into much less viscous, rubbery state.

Figure 4-6: Water vapour uptake at individual RH conditions in the stepwise ramp for amorphous lactose at 25°C.
4.1.3 **The effect of temperature on water sorption by amorphous lactose**

**Figure 4-7:** The effect of temperature on water sorption isotherms of amorphous lactose; arrows indicating the RH corresponding to $W_g^6$ (amount of water required to lower dry $T_g$ to experimental temperature).

<table>
<thead>
<tr>
<th>Temperature $T$ ($°C$)</th>
<th>Weight of water sorbed before onset of crystallisation ($W_{cry}$) (% w/w)</th>
<th>$T_g$ ($^{cry}$) (GT equation) ($°C$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>25</td>
<td>11.9 %</td>
<td>1.1</td>
</tr>
<tr>
<td>30</td>
<td>11.2 %</td>
<td>5.3</td>
</tr>
<tr>
<td>35</td>
<td>10.3 %</td>
<td>10.2</td>
</tr>
<tr>
<td>40</td>
<td>9.6 %</td>
<td>13.9</td>
</tr>
</tbody>
</table>

**Table 4-2:** The amount of water sorbed ($W_{cry}$) required to induce spontaneous crystallisation in amorphous lactose as a function of isothermal temperature; $T_g$ ($^{cry}$) = $T_g$ of amorphous lactose containing amount of water content corresponding to $W_{cry}$.

The effect of temperature on water uptake properties of amorphous lactose is shown in Figure 4-7. Water uptake was least affected at 5%RH with the change in temperature from 25 to 55°C. Beyond 5%RH the sorption lines are separating indicating clear differences in water uptake properties of amorphous lactose with change in temperature. The uptake of water increased with the increase in temperature at the same RH. Whereas the amount of water uptake required for inducing spontaneous

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$^9$ $W_g$ was calculated using Gordon Taylor equation.
crystallisation was lowered with the increase in temperature. Considering the process of water sorption as an exothermic process the amount of water uptake was expected to decrease with an increase in temperature for the same RH and this is widely reported for polymers (Oksanen and Zografi, 1990) and carbohydrates (Hancock and Dalton, 1999). The estimation of equilibrium water uptake value in the mid RH region (25-35%RH) was difficult even after longer exposure time (7h)\(^\text{10}\) at each RH step. Under the time scale of the current experiment, an increase in water uptake with an increase in temperature could be linked to molecular mobility in the amorphous state. It is thus possible to correlate increase in molecular mobility (due to increase in temperature) to increase in water holding capacity of amorphous lactose.

![Figure 4-8](image)

**Figure 4-8:** The extent to which T\(_g\) of the amorphous lactose was reduced below the experimental temperature (T\(_{gcry}\)-T) to induce the spontaneous crystallisation, shown as a function of temperature.

The amount of water sorbed at each temperature to induce the spontaneous crystallisation in the amorphous lactose (W\(_{cry}\)), is shown in Table 4-2. It is not true that amorphous lactose will be unable to crystallise below W\(_{cry}\) but at W\(_{cry}\) the crystallisation response was spontaneous. The value of W\(_{cry}\) decreased with an increase in temperature and this was accompanied with rise in T\(_{gcry}\) of binary mixture predicted using Gordon Taylor equation (Table 4-2). The extent to which the glass transition temperature of binary mixture was reduced below experimental temperature (T\(_{gcry}\)-T) to induce the spontaneous crystallisation in amorphous lactose is shown as a

\(^{10}\) The longer exposure time was not feasible considering the availability of the DVS.
function of temperature in Figure 4-8. $T_{g_{cry}} - T$ remained constant initially over 25-40°C and then started falling over 40-50°C. $T_{g_{cry}} - T$ could be correlated to the barrier for spontaneous crystallisation. Reduction in the values beyond 40°C could mean the reduction in the barrier as the temperature was increased.
4.2 Interaction of water vapour with amorphous indomethacin

Indomethacin being hydrophobic in nature was expected to interact differently with water vapours as compared to amorphous lactose.

4.2.1 Water sorption isotherm for amorphous indomethacin

![Graph showing water uptake vs RH for amorphous indomethacin.]

Figure 4-9: Water vapour sorption isotherm for amorphous indomethacin at 25°C.

The water sorption isotherm for amorphous indomethacin is shown in Figure 4-9. The shape of the isotherm was similar to a type-III BET isotherm. The water sorption behaviour for the amorphous indomethacin was different than that observed for amorphous lactose (which was type-II BET). A type-III isotherm was observed for water sorption by amorphous Glucose at 40°C (Zhang and Zografi, 2000), Tg of dry glucose being 30±4°C. Since amorphous Glucose at -10°C followed the type-II water sorption isotherm it was also concluded that a type-III isotherm would be observed for the amorphous state when the experimental temperature was above the Tg. In this work a Type-III isotherm was observed for amorphous indomethacin at 25°C, Tg in dry state (c.a.48°C) being much higher than the experimental temperature.

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11 Type-III isotherm is exemplified by adsorption of bromine on silica gel at 79°C (Cameron and Reyerson, 1935), silica gel being in the glassy state the curvature in the isotherm was explained to be due to multilayer adsorption and capillary condensation.
The transitions in amorphous indomethacin could not be observed as a result of water uptake from the sorption isotherm at 25°C (Figure 4-9). This was in comparison with water uptake by amorphous lactose, where the spontaneous crystallisation could be clearly followed by a sudden weight loss or desorption of absorbed water. The value of $W_g$ predicted from the Gordon Taylor equation for 25°C was more than 2.5% w/w indicating that the bulk of amorphous indomethacin existed in a glassy state at 90%RH. Although the possibility of a phase transition could be observed for amorphous indomethacin stored at 84.3%RH (Section 3.3.1) from the DSC scans.
4.2.2 Water sorption kinetics for amorphous indomethacin

The dynamically recorded weight change of amorphous indomethacin with a stepwise increment in RH at 25°C is shown in Figure 4-10. The equilibration was observed at all RH steps within about 180 min of exposure. A comparison of water uptake during different RH steps is shown in Figure 4-11 at 25°C. The amount and rate of water uptake at each RH step was higher as compared to the steps before (except for 20%RH where it was lower than 10%RH step). It was possible only when the sorbed water was making the channels for the further uptake of the water. This indicated the diffusivity of the water as a rate limiting step in the water vapour uptake by the amorphous indomethacin. The increase in the step size at each progressive RH indicated the water vapour being distributed from the surface to the core of the amorphous particle. The differential distribution of the sorbed water could also lead to differential plasticisation of the amorphous particle with the surface being plasticised to a greater extent than the core. There were no signs of crystallisation in amorphous indomethacin due to exposure to water vapours over the experimental time scale at 25°C.
Figure 4-11: A comparison of water vapour uptake for different RH steps from the stepwise RH ramp for amorphous indomethacin at 25°C.

The uptake of water vapour at each RH from the stepwise ramp is shown in Figure 4-11. Each line in Figure 4-11 is the water sorption response for a 10%RH increase to the stated RH value (e.g. 20%RH is the step from 10-20%RH). The extent of water uptake at each RH step could be seen from the weight change value at which a plateau was observed. Whereas the rate of water uptake could be seen from the initial curvature of each line, the higher the curvature the faster was the water uptake. It could be seen from Figure 4-11 that the rate and extent of water uptake increased progressively with an increase in RH. The uptake of water vapour at 80 and 90%RH was much higher than water uptake at other RH values.

It was noticed for amorphous indomethacin samples exposed to different RH conditions that at RH ≤ 84% the amorphous state existed as a collapsed state or a rubbery state (section 3.3.1). The higher rate and extent of water uptake at 80 and 90%RH as compared to lower RH values could be linked to the presence the amorphous solid in the rubbery state as opposed to a glassy state.
4.2.3 The effect of temperature on the water sorption by amorphous indomethacin

Figure 4-12: The effect of temperature on the water vapour uptake by amorphous indomethacin.

The effect of temperature on the water sorption isotherm of amorphous indomethacin is shown in Figure 4-12. At 10%RH there was little difference in water sorption over 25 to 45°C but from 20 to 60%RH water sorption followed different trends with changing temperature. For any RH value, water sorption at 25°C was similar to 30°C and at 35°C was similar to 40°C but water sorption at 25 and 30°C was lower than 35 and 40°C and water sorption at 45°C being the maximum. This behaviour was against water sorption norms which follows a decrease in water uptake with the rise in temperature. There could be two reasons for this, one the equilibrium was not reached in the experimental time scale (180 mins) or two the amorphous state of indomethacin was absorbing an increased amount of water with increased temperature due to increased molecular mobility. For 40 and 45°C, the water sorption was lower at 70%RH than the water sorption at 25 and 30°C. At 80 and 90%RH for 40 and 45°C, amorphous indomethacin started loosing water as a sign of crystallisation, the effect was more pronounced at 45°C. At 80%RH, the water uptake at 25 and 30°C was similar to the water uptake at 35°C indicating increased uptake at 25 and 30°C. At 90%RH the water uptake followed the norm with 25°C>30°C>35°C.
Figure 4-13: The water vapour uptake at individual RH conditions in the stepwise ramp for amorphous indometacin.
Figure 4-14: The water vapour uptake at individual RH conditions in the stepwise ramp for amorphous indomethacin.

The water vapour uptake at each RH from the step wise ramp is shown in Figure 4-13 and Figure 4-14. The higher water uptake at 80 and 90%RH (for 25°C) (Figure 4-11) as compared to the water uptake at lower RHs was correlated to the existence of amorphous indomethacin in a rubbery state at ≥80%RH (Section 4.2.1). At 30 and 35°C the uptake of water vapour increased more gradually as compared to 25°C (for
80 and 90\%RH). A gradual increase in water uptake (as seen for 30\(^\circ\)C) is possible when transformation from the glassy to rubbery form is partial or limited to only a part of the solid sample as opposed to a bulk transformation. As amorphous indomethacin is hydrophobic, a uniform distribution of water absorbed water vapour may not be possible. Since water uptake should take place from the surface of a particle there could be localization of sorbed water at the surface. In such a scenario it could be possible that the surface of particle getting plasticised preferentially rather than the whole bulk and the process thus initiated at the surface would continue slowly to the bulk. The preferential surface plasticisation is more pronounced when temperature difference between \(T_g\) and \(T\) is lower, where a small amount of water absorbed could lower the \(T_g\) to \(T\). The rise in experimental temperature from 25\(^\circ\)C to 35\(^\circ\)C for amorphous indomethacin lowered \(T_g-T\) from about 20 (at 25\(^\circ\)C) to 10\(^\circ\)C (35\(^\circ\)) hence the small amount of water sorbed at the surface could easily lower \(T_g\) to \(T\). The gradual plasticisation from surface to bulk could lead to water sorption isotherm devoid of any sudden changes in weight gain at a particular RH.

Although there were noticeable differences in water sorption behaviour of amorphous indomethacin among individual RH and temperature conditions it was difficult to define the conditions for plasticisation and transition into the rubbery state as there were no obvious break points in the isotherm.
4.3 Interaction of water vapour with amorphous nifedipine

The uptake of water vapour by amorphous nifedipine (quench cooled) is shown in Figure 4-15. It could be seen that from 0-50% RH, the water vapour uptake increased gradually but after 50% RH it was slowed down (Figure 4-15). The slowing down of water vapour uptake beyond 50% RH could be due to crystallisation of amorphous particles at the surface, the crystals formed at the surface covers the amorphous core. The surface crystallisation of amorphous terfenadine has been reported (Samra and Buckton, 2004); the crystallised form at the surface blocked the further ingress of water molecules and thus prevented crystallisation at the core of a particle.

![Figure 4-15](image)

Figure 4-15: The water vapour sorption of amorphous nifedipine (quenched cooled) at 25°C.

A dynamically recorded water sorption profile for the spray dried (SD) amorphous nifedipine is shown in Figure 4-16. The shape of water sorption isotherm observed for SD nifedipine at 20°C was similar to type-III isotherm (Figure 4-18). A comparison of water sorption for SD nifedipine at individual RH step is shown in Figure 4-17. The amount of water absorbed at each RH step started increasing beyond 40% RH and reached a maximum value at 90% RH.
Figure 4-16: The water vapour sorption of spray dried amorphous nifedipine at 20°C.

Figure 4-17: The water vapour uptake at individual RH conditions in stepwise ramp for spray dried amorphous nifedipine at 20°C.
The water uptake at 60 and 70%RH could not reach the equilibrium value, indicating a sorbed water induced transition in SD amorphous nifedipine, as discussed for amorphous lactose. The water vapour sorption at 20°C did not show any indication of crystallisation in SD nifedipine as the sample did not show any loss of the sorbed water.

**Figure 4-18:** The effect of temperature on the water vapour uptake of spray dried amorphous nifedipine.

The effect of temperature on water vapour uptake by SD nifedipine is shown in Figure 4-18. Water vapour uptake for SD nifedipine decreased with increase in temperature, this may be due to aging of the amorphous state. It could be recalled from section 3.4.1 that amorphous nifedipine exhibited very short relaxation time near the glass transition temperature. Aging of the amorphous solid caused reduction in free volume and hence can potentially reduce water sorption potential of amorphous solids. In the case of amorphous trehalose the aging process caused reduction in the amount of water uptake, although beyond a certain RH it sorbed the same amount of water irrespective of the extent of aging (Surana et al., 2004a). The aging in SD nifedipine was irreversible or could not be erased due to water sorption as observed for water sorption of trehalose.
4.4 A comparison of water vapour sorption by amorphous lactose, indomethacin and nifedipine

Although amorphous, the three compounds (lactose, indomethacin and nifedipine) exhibited different water uptake and holding properties. Although the BET equation cannot theoretically be applied to water sorption isotherm of amorphous solid, amorphous lactose exhibited apparent type-II whereas amorphous indomethacin and nifedipine exhibited apparent type-III isotherm. The sorbed water induced transitions in amorphous lactose (e.g. plasticisation, collapse and crystallisation) which could be followed from, water sorption isotherm. Although such transitions cannot be denied in amorphous indomethacin they were not as obvious from water sorption isotherm. The sorbed water induced spontaneous crystallisation in amorphous lactose; this was not the case with amorphous indomethacin. Water vapour exposure of amorphous indomethacin at higher temperatures (e.g. 40 and 45°C) was required to induce observable crystallisation. In the case of amorphous indomethacin absorbed water induced a slow crystallisation process which may take several hours to days for completion.

The differences in water vapour interaction of amorphous lactose and indomethacin could be the distribution of the sorbed water. Whereas absorbed water distributes freely throughout the bulk of the solid in the case of amorphous lactose, it may be localised preferentially at the surface for amorphous indomethacin, this differential water distribution may lead to only a part of the solid undergoing transitions as opposed to the bulk transitions.

At any given RH, the amount of water uptake was increased with an increase in isothermal temperature for amorphous lactose and amorphous indomethacin. This effect was attributed to an increase in molecular mobility with the increase in the temperature. In the case of amorphous nifedipine increasing the isothermal temperature led to a decrease in water uptake at all RH values.
Chapter 5  Microcalorimetric studies on the interaction of solvent vapour with amorphous solid
5.0 Microcalorimetric studies on the interaction of solvent vapour with amorphous solid

In the previous section, the interaction between amorphous solid and water vapours was studied gravimetrically (Section 4.0). In this part of thesis, the interaction between amorphous solids and solvent vapour is reported using calorimetry, which detects the heat flow generated as the amorphous solids are exposed to solvent vapour.

5.1 Theoretical background for calorimetric studies

When the solid surface is exposed to solvent vapour, the process of adsorption of vapour on the solid surface takes place, which can be expressed as follows,

\[ A \xrightarrow[\sigma]{} B \]

Where A is the number of active sites on the solid, G is the concentration of solvent vapour and B is the number of sites on the solid surface occupied by vapour molecules. The heat exchange due to this reaction can be expressed by equation 22.

\[ \frac{dQ}{dt} = -\Delta H \frac{db}{dt} \]

Equation 21

Where \( \Delta H \) represents heat of formation of B and \( \frac{db}{dt} \) is a kinetic parameter which represents the rate of reaction. According to equation 22 the power output recorded would be proportional to the rate of reaction with the heat of reaction being a constant parameter. For exothermic reactions heat will be released and \( \Delta H \) will be negative, generally a bond formation process is considered to be exothermic (e.g. adsorption of gases on a solid surface and transformation of amorphous state into crystalline state). On the other hand endothermic reactions will have an intake of heat from the surroundings and \( \Delta H \) value would be positive, the bond breaking process is considered as endothermic (e.g. de-sorption of gases from a solid surface).

The equation 22 could be elaborated as equation 23 (Bakri and Lechuga-Ballesteros, 1999).

\[ P = \frac{dQ}{dt} = \Delta H k \left[ (n-1)kt + (A_0)^{1-n} \right]^{\frac{n}{1-n}} \]

Equation 22

Where P is the power output at time t, n is the order of reaction (n=1,2,3,...), k is the reaction rate constant and \( A_0 \) is the number of active sites available at time t=0.
For zero order reaction equation 23 could be expressed as equation 24.

\[ P = -\Delta H k_0 \]  
\textbf{Equation 23}

Where, \( k_0 \) is the zero order rate constant.

For a zero order reaction, the rate of reaction is independent of number of active sites hence the power output signal would be a straight flat line as shown in Figure 5-1B.

Whereas in the case of first order reaction equation 23 could be expressed as equation 25.

\[ P = \Delta H k_1 A_0 e^{-k_1 t} \]  
\textbf{Equation 24}

Where, \( k_1 \) is the first order rate constant.

Initially the power output signal would be high, due to the large number of reactive sites available, which then decays exponentially over a time period. The shape of the power output signal obtained for a first order reaction could be similar to Figure 5-1A.

\textbf{Figure 5-1:} The shape of power output signal obtained by a calorimeter for first (A) and zero (B) order reactions. The different lines in A exemplify the different shapes due to different \( A_0 \) and \( k_1 \); whereas the different lines in B exemplify differences only due to different \( A_0 \).

Although the kind of reaction (zero order, first order etc.) can be estimated from the power output data, it is not unusual to encounter multiple parallel reactions (different reactions occurring at the same time), chain reactions (product of first reaction being the reactants for second reaction) or change in the order of the reaction at some time point etc. The power output signal in such cases can show much complex changes and hardly give any information about the kind of reaction taking place. The major drawback of the calorimetric studies is that it records total heat and is non-specific about the kind of the reaction taking place Buckton 1999.
5.2 Interaction of water vapour with amorphous lactose

The gravimetric water sorption studies indicated complex interactions between water vapour and amorphous lactose. The power output signal obtained by a serial ramp of RH (3%RH/h) for amorphous lactose indicated a few clear transitions (Figure 5-2). Initially power output was exothermic which increased rapidly over the first 10%RH after that it remained steady until about 25%RH. At 27.5%RH power output became gradually more exothermic until about 48%RH after which it decreased slightly and then constant power output was observed over the next 10%RH. At 60%RH power output started decreasing and then formed a plateau until 67%RH after which it increased and then decreased rapidly giving a sharp peak. The decrease in power output signal continued until it became endothermic.

![Figure 5-2:](image)

Figure 5-2: The power output signal obtained by a serial ramp of RH (3%RH/h) over amorphous lactose. Arrows indicate RH at which power output signal changed indicating possible transitions in amorphous solid.

A similar moisture induced thermal activity traces (MITAT) for sucrose, lactose, raffinose and sodium indomethacin were reported before using a serial RH ramp (Lechuga-Ballesteros et al., 2003). These authors classified the trace into different regions (Figure 5-2) a low activity region, A; a take-off point, B; a high exothermic activity region, C and an endothermic region, D. When samples of amorphous sucrose were stored in the low thermal activity region of RH (Region A) they remained unchanged for more than three years but when stored at RH above the take off point (Region B) they did undergo a slight collapse. Whereas gross structural changes and crystallisation were reported in the amorphous state stored at RH corresponding to maximum thermal activity (C). The value of RH corresponding to the take off point (B)
was taken as RHm and the point of maximum exothermic activity as RHp. The value of RHm was also correlated to a limit of hydration for the amorphous state or to the point beyond which water activity increased. It could also be recalled from the gravimetric studies (Section 4.1.1.1) that RHm was the region of RH where monolayer volume coverage (BET theory) was observed for amorphous particles.

The take-off point (B) could be correlated to a point where the internal structure of the amorphous particles started changing and hence to a point of mobility onset in the amorphous solid (RHm).

Figure 5-3: A comparison of power output signal obtained by a serial ramp of RH (2%RH/h) over amorphous lactose with gravimetric data obtained by a similar experiment using DVS (from Burnett et al., 2004). Arrows indicate water sorption behaviour corresponding to take off point (B) and the maximum exothermic point (C). Wg is the amount of water required to lower dry Tg to 25°C, calculated using Gordon Taylor equation.

It was interesting to know whether the rise in power output signal at RHm was due to a change in the amorphous state of lactose (induced by absorbed water) or due to a change in mode of water uptake (i.e. from surface adsorption to bulk absorption). A comparison of power output signal with gravimetrically recorded water uptake profile is displayed in Figure 5-3. The water uptake profile of amorphous lactose changed drastically in the take-off region (from power output signal Region B) which was correlated to RHm. Although the take off point (B) in power output signal could correspond to a rise in water uptake rate (hence to a rise in exothermic response), the possibility of any physical transitions in amorphous lactose (in this RH region) could
not be excluded. It was difficult to estimate the changes in the physical state of the amorphous solid from power output or gravimetric weight change data individually. Also shown in Figure 5-3 is the amount of water required to be sorbed ($W_g=7.94\%w/w$) in order to reduce $T_g$ of dry amorphous lactose to the experimental temperature ($25^\circ C$) and RH corresponding to $W_g$ ($RH_g=41.9\%$). $RH_g$ was in between $RH_m$ (take of point, point B) and $RH_p$ (maximum exothermic activity, point C) and there was no sudden change in water sorption properties or power output signal at $RH_g$.

![Figure 5-4: Power output obtained by exposing the amorphous lactose to a stepwise ramp of water vapour pressure (RH) in which the RH was increased in the steps of 5\%RH and held constant for 6h before going to the next step.](image)

To gain more insight about the transitions in take off point region (point B) and the region of maximum exothermic activity (point C) another experiment was carried out in which the RH was increased in the steps of 5\% and kept constant for 6h, corresponding power output and weight change was recorded using TAM and DVS respectively. The power output signal obtained by exposing amorphous lactose to a stepwise ramp of RH is shown in Figure 5-4. For each step change in RH, the power output signal reached a maximum exothermic value almost instantaneously and then started decaying exponentially (shape similar to first order reaction Figure 5-1A). Although a baseline was not reached over 6h of equilibration time it could be anticipated within the next couple of hours. A similar power output signal was observed over step changes between 5-25\%RH. Beyond 25\%RH the shape of the exothermic response was entirely different than that observed until 25\%RH. At 30\%RH, the decay in exothermic power output was much slower and at the end of 6h
did not reach the baseline. At 35%RH the decay in exothermic signal was clearly
superimposed by a shoulder. A similar shoulder in the power output signal could also
be observed at 40%RH. A shoulder in the power output signal was observed in RH
region where it started increasing (RHm to RHp) during the serial RH ramp.

During all RH steps, the amount of water vapour available for amorphous lactose was
kept constant (constant RH) hence the decay in power output was independent of RH.
Hence it was possible to correlate the availability of the active, water binding sites on
amorphous lactose to be the rate limiting step in the water uptake process observed
during stepwise RH ramp. The sorption process at initial steps in the RH ramp (5-
25%RH) could be assumed to be a pseudo first order reaction; hence the rate of
reaction is determined by the number of water binding sites available on the
amorphous lactose. Beyond 25%RH, the shape of the power output signal became too
complex to make any estimate about the kind of reaction taking place. Since the rate
of the reaction (and hence the power output signal) was not influenced by the
availability of water vapour, the appearance of a shoulder at 35 and 40%RH could be
correlated to the changes in internal structure of amorphous state. These changes in
amorphous state increased the number of water binding sites available and hence
resulted in a shoulder on the power output signal.

Water vapour sorption by amorphous lactose at 35%RH step at 25°C did not reach
the equilibrium in 6h but the derivative of this line (the rate of the water vapour sorption,
dm/dt) was also associated with a shoulder as observed for the power output signal
(Figure 5-5A). The dm/dt line for 40%RH was also associated with a similar shoulder
as observed for power output signal at 40%RH, whereas dm/dt lines for 15 and
25%RH followed an exponential decay (Figure 5-5B). The shape of the dm/dt line at
RH steps matched with the shape of the power output signal at respective RH values
(Figure 5-5A). Although the two instruments used (TAM and DVS) were having
different sensitivity (limit of detection) the shape of power output signal obtained using
TAM followed very similar changes to rate of water vapour uptake (dm/dt) profile
obtained from DVS. Changes in the power output signal (in serial ramp and step
ramp) in the 30-45%RH were mainly related with changes in the rate of water vapour
uptake. These results match with reported studies for water vapour interaction of
amorphous sucrose studied using the double twin isothermal calorimeter (DTIC)
(Lechuga-Ballesteros et al., 2003). The authors assumed that the heat of interaction
between amorphous solids and water vapour was the same as the heat of
condensation of water vapour (under atmospheric pressure). Using DTIC, the total
power output signal was de-convoluted into a power output signal due to water
sorption and that due to all other processes that do not involve sorption or desorption
of water.
**Figure 5-5:** The rate of water vapour uptake by amorphous lactose at 25°C; A. at 35%RH; B. a comparison between different RH steps. Arrows indicate the position of shoulder on dm/dt line (dt=5 min.).
The authors found that the power out signal in the low exothermic activity region (A), take off point (B) and region C (Figure 5-2) was entirely due to the vapour sorption. It was only during region D, where other processes became apparent and were related to water induced collapse of the glassy phase. The inability to detect transitions in region A-C does not necessarily mean absolute absence of any transitions but could be due to the insensitivity of DTIC, to deconvolute extremely small signals.

In an attempt to study this issue further, total heat released during each stepwise increase in RH was integrated from the power output signal and the corresponding amount of water sorbed at each step was estimated from DVS for amorphous lactose at 25°C. The two experiments were then combined in order to calculate the total amount of heat released during one mole of sorbed water at each RH step. Total heat released (ΔH) (Figure 5-6) for each RH step was lower than the heat of condensation of water (assuming water sorption process to be similar to the condensation of water). The value of ΔH decreased gradually from 15%RH and was lowest at 35-45%RH after which it increased again at 50%RH to a value near the heat of condensation of water (44 kJ/mole).

**Figure 5-6:** Total heat released (ΔH) during water uptake at each RH step by amorphous lactose (at 25°C), calculated by integrating individual exothermic response from Figure 5-4, the amount of water uptake at each RH step was taken from the similar DVS experiment. The heat released during condensation of water at 25°C under atmospheric pressure was taken as 44 kJ/mole.

Considering the error associated with integration of power output signal ΔH values reported here cannot be taken as an absolute value (due to the lack of the
equilibration under the experimental time scale) but could be used confidently to differentiate between water uptake process during various RH steps. At low RH (10-15%RH), the value of ΔH was closer to 44 kJ/mole (heat of condensation of water) indicating that the sorption process was mostly associated with formation of hydrogen bonds with solids (Figure 5-6). Whereas the gradual decrease in ΔH values over 15-35%RH indicated the presence of parallel reaction which, compensated the exothermic ΔH values. It was obvious that water sorption process in this RH region was accompanied with changes in internal structure of amorphous state (e.g. breaking of bonds between molecules in amorphous state). Since breaking of the bonds between molecules is accompanied with an uptake of heat, the total ΔH value for water sorption process would be lowered. The extent of lowering of ΔH below 44 kJ/mole indicated the extent of deformation in internal structure of amorphous state; hence water vapour induced deformation was the maximum during 35-45%RH. The take off point (27%RH) in power output signal obtained by a serial ramp of RH was in the region where ΔH values started decreasing gradually. The region of maximum exothermic activity (47%RH) was in RH range of minimum ΔH values or maximum internal structural changes in the amorphous lactose were observed.

It could be concluded from above discussion that the take off point (point B) in power output signal from RH ramp for amorphous lactose corresponds not only to an increase in rate of water vapour uptake but also corresponds to onset of internal structural changes in amorphous state.
5.3 Interaction of amorphous indomethacin with water vapour

The power output signal obtained by exposing amorphous indomethacin to a serial ramp of RH was exothermic and increased very slowly over 10-40%RH, and then remained constant over 40-65%RH (Figure 5-7). Beyond 70%RH the power output started increasing rapidly and then decreased giving a complete peak; over 80-90%RH the power output increased gradually.

![Graph showing power output signal and RH over time](image)

**Figure 5-7:** The power output signal recorded by exposing amorphous indomethacin to a serial ramp of RH (3%RH/h), arrow indicates a transition point in amorphous state.

Experiments involving a serial ramp are associated with gradual increase in relative pressure of solvent vapour (hence solvent vapour pressure is not kept constant). Since the process of vapour sorption is also rate controlled by the number of solvent vapour binding sites available on the solid, solvent vapour uptake by amorphous solid could be considered as at least second order process. Assuming that the interactions between the solid and solvent vapour proceeds without any change in physicochemical properties of the solid, the number of solvent binding sites would decrease and solvent vapour pressure would increase as the experiment proceeds.

Assuming that the heat of vapour sorption remained constant over the entire sorption process (or RH range), the initial slow increase in power output (over 10-40%RH) could be due to an increased rate of the water uptake with the rise in RH. A constant power output signal over 40-65%RH range could be associated with constant rate of water
vapour uptake (apparent zero order reaction). The rise in power output beyond 70%RH could be due to a rise in rate of water vapour uptake which continued until 95%RH. The overlaying peak in power output signal could be either due to a rapid rise and fall in the rate of water vapour uptake and/or the relaxation of the amorphous indomethacin due to the absorbed water. Similar transitions in the rate of water vapour uptake were observed for amorphous indomethacin by a serial ramp in RH as shown in Figure 5-8. Water vapour uptake was comparatively much higher during 80 and 90%RH steps at 25°C (Figure 4-11).

Figure 5-8: The water vapour uptake and the rate of the uptake, dm/dt (derivative plot dt=1.8min.) obtained by a serial ramp of RH (at 3%RH/h) for amorphous indomethacin at 25°C.

The rise in power output for amorphous indomethacin at 70%RH was similar to the take off point as observed for amorphous lactose using water ramp c.a. 27%RH (Figure 5-2). The important difference between the dry amorphous state of lactose and indomethacin was the molecular mobility at 25°C. Amorphous indomethacin exhibited significant molecular mobility in the dry state at 25°C, with the average relaxation time c.a. 34h, whereas no molecular mobility was detected in amorphous lactose below 60°C (Section 3.4.3). Generally, the relaxation time decreases as temperature approaches dry Tg. At Tg the average relaxation time is approximated as 100 sec (Ediger et al., 1996). Since sorbed water is known to reduce Tg, the relaxation time would decrease with an increasing amount of sorbed water. At a certain amount of sorbed water (Wg) dry Tg would be lowered to the experimental temperature (25°C) and the relaxation time of molecules in the glassy state would fall in the experimental time
scale (or amorphous state would undergo a glassy to rubbery transition). The relative humidity corresponding to $W_g$ could be regarded as $R_{Hg}$ which would be critical for a particular amorphous state at a specific temperature. The HDSC studies performed on amorphous indomethacin exposed to different RH conditions also indicated that at storage beyond 84%RH, the amorphous state existed as a relaxed state (Section 3.3.1).

![Figure 5-9: The power output signal obtained by exposing dry amorphous indomethacin to alternating conditions of the RH (0-70-0-80-0-75-0-80%RH), with 6h at each step. The arrows show shoulder in power output signal.](image)

When dry amorphous indomethacin was exposed to 70%RH the power output signal rapidly increased to a maximum and then started decaying towards the baseline but when it was exposed to 80%RH (Figure 5-9) or 85%RH (Figure 5-10) the power output increased to the maximum and started decaying with a shoulder. The shoulder obtained on the MITAT was in the RH region where the peak in MITAT was observed during the serial RH ramp and could be due to two reasons. One, due to a change in rate of water sorption behaviour of amorphous indomethacin and second due to an occurrence of second reaction running parallel to water uptake (e.g. relaxation of glassy, amorphous state or crystallisation). The shoulder could not be correlated to a crystallisation process since amorphous indomethacin at 84%RH and 30°C took about 60 days to recrystallise completely (Andronis et al., 1997), the time scale comparatively longer than the experimental time scale.
Figure 5-10: The power output signal obtained by exposing dry amorphous indomethacin to 85%RH for 37h and then 0%RH.

Figure 5-11: The rate of water vapour sorption by amorphous indomethacin at 25°C; A. at 80%RH; B. Comparison at different RH steps, (dt=5 min.).

The shoulder obtained in MITAT for 80%RH could also be seen for second 80%RH exposure with the 0-75-0%RH treatments in between (Figure 5-9). The heat released during water vapour sorption at 85%RH was almost equal to the heat uptake during de-sorption at 0%RH (Figure 5-10), indicating that the major component of heat was due to reversible reaction/s. The shoulder in MITAT for amorphous lactose was associated clearly with a change in rate of water sorption behaviour. The rate of water sorption for amorphous indomethacin at 85%RH was not associated with a clear shoulder (Figure 5-11) but a deviation from exponential decay for rate of water uptake could be clearly seen. The MITAT obtained from TAM clearly amplified the change in
water interaction behaviour of amorphous indomethacin at 85%RH as compared to water sorption behaviour obtained from DVS.

It was evident from water vapour and amorphous lactose interaction studies that the increase in exothermic signal at take off point was not only related to an increase in rate of water vapour sorption by lactose but was also associated with changes in internal structure of amorphous lactose induced by absorbed water vapour. Although amorphous indomethacin exhibited significant molecular mobility in the dry state at 25°C it was possible to correlate the take off point observed in MITAT from serial RH ramps to the point where significant changes in internal structure took place.

![Graph](image)

**Figure 5-12:** Estimation of critical RH to induce transitions in the amorphous indomethacin from the derivative of power output signal obtained by serial ramp of RH at 25°C.

The RH corresponding to the points at which take off in MITAT signal was observed could be estimated from the derivative of power output signal (Figure 5-12). Three points were recognized to characterise the transition. The first point (1) where MITAT actually started increasing considerably as seen from the deflection of dP/dt line from baseline (71.5%RH); the second point (2) where the rate of increase or the slope of the MITAT was maximum, corresponding to the peak of the dP/dt line (76.6%RH) and the third point (3), where MITAT started decreasing corresponding to a point of intersection between baseline and dP/dt (77.6%). As mentioned previously all three critical RH values were in the region where amorphous indomethacin was observed to be collapsed as seen from the Hyper-DSC analysis (section 3.3.1).
Figure 5-13: Estimation of the critical RH (RH corresponding to the time point) to induce transitions in the amorphous indomethacin from the derivative of the power output signal obtained by serial ramp of the RH at 35°C.

Figure 5-14: Estimation of critical RH to induce transitions in amorphous indomethacin from derivative plot of power output signal obtained by a serial ramp of RH at 40°C.

The MITAT obtained by the serial ramp of RH for amorphous indomethacin at 35°C (Figure 5-13) was similar to the one obtained at 25°C. The MITAT obtained at higher
temperature, 40°C (Figure 5-14) was more complex as compared to lower temperatures, which may be due to different amorphous regions relaxing at slightly different RH. The possibility of partial crystallisation in amorphous regions at high RH was evident from water vapour sorption studies at 40°C (Section 4.2.3).

\[ \text{Figure 5-15: The values of critical RH obtained from MITAT for amorphous indomethacin as a function of temperature.} \]

The different RH values corresponding to the different points obtained from the MITAT at different temperature obtained are shown in Figure 5-15. It could be seen that although the %cRH values corresponding to point 1 decreased with the increase in temperature there was no direct temperature dependence was observed for point 2 and 3.
5.4 Interaction of amorphous indometacin with organic vapour

Like absorbed water, organic solvents can also act as a plasticizer for the amorphous state. In this section of the thesis, the interaction of amorphous indometacin with organic solvent vapours (mainly aliphatic short chain alcohols) at various relative pressures is reported using perfusion calorimetry.

5.4.1 Interaction of ethanol vapours with amorphous indometacin

![Graph](image)

Figure 5-16: The power output signal obtained by a serial ramp of relative pressure of ethanol (3%RE/h) for amorphous indometacin (30.07mg) at 25°C. Arrows indicate different regions of the power output trace.

In an experiment similar to the serial water ramp (sections 5.2 and 5.3), amorphous indometacin was exposed to a serial ramp of relative pressure of ethanol (%RE). The power output signal was variable over the range of %RE indicating the different transitions in amorphous indometacin (Figure 5-16). The power output was initially exothermic (about 5μW) and was almost constant over 0-15%RE (although slightly more exothermic at around 12%RE, Region-A). Just below 15%RE, the power output started slowly decreasing (less exothermic) and remained constant (at around 0μW) until c.a. 50%RE. The power output started increasing beyond 50% RE rapidly (more exothermic) and this continued until 95%RE. Previous experiments demonstrated that amorphous indometacin crystallised when exposed to saturated ethanol vapours making it difficult to interpret the power output signal. The power output signal obtained by exposing amorphous indometacin to ethanol vapours in a sealed ampoule experiment of TAM is shown in Figure 5-17. Under the atmosphere of saturated
ethanol vapours amorphous indometacin crystallised completely. This indicated that the power output signal recorded by TAM was due to crystallisation of amorphous solid. Ethanol induced crystallisation of a hydrophobic drug has been previously shown in our lab using TAM (Ahmed et al., 1996).

It was clear that ethanol vapours were absorbed into amorphous indometacin during the serial ramp; due to its low Tg, about 97.2 K (Lesikar, 1975) ethanol lowered Tg of amorphous solid and thereby induced crystallisation. It was interesting to observe a couple of points from the serial ramp (Figure 5-16) first, %RE which would lower dry Tg of amorphous indometacin to experimental temperature(%cREg) and second %RE which would induce spontaneous crystallisation in the plasticised state (%cREcry). The process of plasticisation induced by ethanol vapour may not be uniform throughout the bulk of the powder and may lead to predominant surface plasticisation and hence surface crystallisation.

A comparison of two crystallisation events, water vapour induced crystallisation in amorphous lactose and ethanol vapours induced crystallisation in amorphous indometacin has demonstrated interesting differences (Figure 5-17).

![Figure 5-17](image)

**Figure 5-17:** The crystallisation response obtained for A. amorphous indometacin exposed to the saturated ethanol vapours B. amorphous lactose to exposed to 75%RH (Dilworth et al., 2004) at 25°C in a sealed ampoule experiment of TAM. The arrow indicates the time-point of lowering an ampoule to the measuring position and the asterisk indicated the crystallisation response.

Firstly the rate of water vapour availability was the decisive factor in onset of crystallisation of lactose (about 2.5 h of lag time between water vapour exposure and crystallisation), this was not the case for ethanol vapour induced crystallisation in amorphous indometacin. One reason for this behaviour could be the higher vapour pressure ($p^0$) of ethanol (58.71 torr) as compared to vapour pressure of water (23.77 torr) at 25°C (Plambeck, 1996), this could also mean that evaporation of ethanol could be expected to be much faster as compared to evaporation of water at 25°C.
Irrespective of the reason, it was clear that certain amount of ethanol vapour pressure induced crystallisation in amorphous indometacin almost instantaneously.

**Figure 5-18:** Ethanol vapour uptake by amorphous indometacin (32.4 mg) at 25°C studied by the serial ramp of the partial pressure of the ethanol vapour from 0 to 45%. $\frac{dm}{dt}$ was the derivative of the weight change or the rate of uptake $dt=10$ min.

This fact was almost confirmed from the power output trace obtained by a serial ramp of ethanol vapour pressure for amorphous indometacin at 25°C (Figure 5-16). The power output signal remained almost constant around 0 $\mu$W from about 20%RE to 50%RE, indicating the absence of any reaction over this range of ethanol vapour pressure or ethanol vapour below 50% partial pressure could not induce crystallisation in amorphous indometacin instantaneously. Beyond 50%RE, the power output started rising (increasingly exothermic), this could be due to crystallisation of the amorphous solid. Since it is well documented that the crystallisation is followed by desorption of absorbed vapours, the power output signal was expected to become endothermic with the onset of crystallisation (as observed for crystallisation of amorphous lactose) (5.2). The absence of an endothermic response could be due to lack of sufficient amount of absorbed ethanol vapour which could desorb after crystallisation.

Ethanol vapour uptake by amorphous indometacin studied gravimetrically (Figure 5-18) by a serial ramp of ethanol vapour pressure at 25°C could be correlated with the power output signal (Figure 5-16). Weight gain was rapid initially over 0-25%RE and
then was remained constant over 25-35%RE. At 35%RE amorphous indomethacin absorbed about 0.62% of dry weight of solids and beyond which it started crystallizing. According to the Gordon Taylor equation 0.62% w/w of absorbed ethanol (assuming uniform distribution) would lower the dry Tg of amorphous indomethacin from 320 to 312 K (using the parameters in Table 5-1:).

<table>
<thead>
<tr>
<th>Amorphous indomethacin</th>
<th>Tg (K)</th>
<th>Density (kg/m³)</th>
<th>K</th>
</tr>
</thead>
<tbody>
<tr>
<td>320</td>
<td>1.32</td>
<td>0.181</td>
<td></td>
</tr>
<tr>
<td>Ethanol</td>
<td>97.2</td>
<td>0.785</td>
<td></td>
</tr>
</tbody>
</table>

**Table 5-1:** The parameters used in calculating Tg of binary mixture of amorphous indomethacin and ethanol.

**Figure 5-19:** The power output signal obtained by exposing dry amorphous indomethacin to alternating conditions of RE (0-30-0-50-0-70-0-90-0-90%RE), 6h at each step.

The power output signal obtained by exposure of amorphous indomethacin to a series of ethanol vapour pressures indicated a few changes in the amorphous state (Figure 5-19). For the first 30%RE exposure, the amount of heat released (exothermic) during sorption was more than the heat uptake (endothermic) for next desorption step, this was indicative of a degree of irreversibility in the sorption process. For the second 50%RE step, the heat exchange was almost equal and opposite for sorption and desorption steps. During the third 70%RE step, the power output signal was clearly associated with a shoulder and could not reach the baseline within 6h, this was
indicative of onset of second parallel reaction (possibly onset of crystallisation\textsuperscript{12}). The power output signal at 90\%RE step was in particular the characteristic of the crystallisation event. Repeating exposure to 90\%RE did not repeat the power output signal.

The stepwise exposure in TAM (Figure 5-19) and the gravimetric studies (Figure 5-18) could support the fact that the increasingly exothermic power output signal beyond 50\% partial pressure of ethanol (%RE) (Figure 5-16) was due to the onset of the crystallisation process induced in amorphous indomethacin. Hence 50\% could be regarded as %cRE (critical RE to induce crystallisation) for amorphous indomethacin at 25°C. The initial exothermic response over 5-15\%RE could be linked to the increasing rate of ethanol vapour uptake. This response was similar to take off point (B) observed for amorphous lactose (Figure 5-2) and hence could be correlated to onset of internal changes (induced by ethanol vapour).

\textsuperscript{12} Since the heat exchange for sorption step at 70\%RE was irreversible or more than the heat exchanged during desorption step.
5.4.2 Interaction of n-propanol vapours with amorphous indomethacin

In an experiment similar to the one performed using water and ethanol vapours, amorphous indomethacin was exposed to a serial ramp of propanol vapour pressure (%RP), the recorded power output signal is shown in Figure 5-20. Initially the power output signal was exothermic and remained constant until 10%RP (Point A), then started increasing (became more exothermic) (Point B) and reached the maximum value at 19.3%RP (Point C). Beyond 20%RP, the power output signal decreased slowly but remained exothermic and then kept rising at a constant rate until 62.5%RP (Point D). At point D, the power output signal started rising rapidly and then decreased.

The sequence of changes observed in the power output signal was very similar to the sequence of changes observed during the serial water ramp for amorphous lactose (Figure 5-2). The point B (take off point) and point D for amorphous indomethacin (Figure 5-20) are equivalent to the take off point and point D in the water vapour ramp for lactose respectively.

![Graph](image)

**Figure 5-20:** The power output signal obtained by a serial ramp of relative pressure of propanol (3%RP/h) for amorphous indomethacin (33 mg) at 25°C.

The power output signal obtained by exposure of amorphous indomethacin to stepwise increments in %RP (Figure 5-21) demonstrated transitions similar to those for amorphous lactose exposed to stepwise increment RH ramp (Figure 5-4). The power output signal at 10%RP was clearly associated with a shoulder (Figure 5-21) and this was in the region where the take off point (10.9%RP) was observed with a serial ramp.
At the 60%RP step, the power output signal clearly deviated from the one observed for a typical first order reaction (exponential decay) (shown by asterisk in Figure 5-21), which progressed into a clear shoulder at 80%RP. At the 90%RP step, the power output signal increased to an exothermic value and then suddenly started decreasing until it became endothermic.

At this point it was very clear that the take off point at 10.9%RP in (Figure 5-20) could be due to increased rate of the propanol vapour uptake by amorphous indomethacin and hence could mean that the absorbed propanol induced some changes in internal structure of amorphous indomethacin. Point D (62.5) could be correlated to %RP where amorphous indomethacin started crystallizing rapidly.

**Figure 5-21:** The power output obtained by exposing amorphous indomethacin (35.8 mg) to a stepwise ramp of n-propanol vapour pressure (RP) in which RP was increased in steps of 5% and held constant for 6h before going to the next step.

At this point it was very clear that the take off point at 10.9%RP in (Figure 5-20) could be due to increased rate of the propanol vapour uptake by amorphous indomethacin and hence could mean that the absorbed propanol induced some changes in internal structure of amorphous indomethacin. Point D (62.5) could be correlated to %RP where amorphous indomethacin started crystallizing rapidly.
5.4.3 Interaction of methanol vapour with amorphous indomethacin

Figure 5-22: The power output signal obtained by a serial ramp of relative pressure of methanol (3%/h) for amorphous indomethacin (52 mg) at 25°C.

A serial ramp of methanol vapour pressure over amorphous indomethacin was clearly associated with a series of transitions (Figure 5-22). In the region A power out remained unchanged until 11%RM (Relative pressure of methanol). At point B (11.13%) power out exhibited a positive deflection and kept rising until it reached (Point C) 16.96% after which it started falling. The decrease in power output beyond Point C continued till it became endothermic. At point D (34.36%) power output started increasing again and then became exothermic. From the understanding of water vapour induced transitions of amorphous lactose, a couple of transitions could be followed from power output obtained by a serial ramp of methanol vapour over amorphous indomethacin. Point B in Figure 5-22 could be associated with a rise in rate of methanol vapour uptake by amorphous indomethacin but could also be indicative of internal structural changes induced by methanol. Point C (16.96%) where the power output reached a peak could be correlated to the point where the sorbed methanol reduced Tg of amorphous indomethacin to experimental temperature. Point D could be linked to the point where sorbed methanol induced crystallisation in amorphous indomethacin (since the power output signal became increasingly exothermic and then endothermic).
Point C and point D could be correlated to critical methanol vapour pressure (%cRM) for amorphous indomethacin at 25°C. %RM at point C could be correlated to a relative pressure of methanol required to lower Tg of dry amorphous indomethacin to 25°C, hence %RM at point C could be correlated to %cRMg. Whereas %RM at point D could be correlated to a relative pressure of methanol required to induce crystallisation in amorphous indomethacin, hence %RM at point D could be correlated to %cRMcry for amorphous indomethacin at 25°C.
5.4.4 Effect of temperature on interaction of amorphous indomethacin with methanol vapour

![Graph showing power output signal vs time and temperature effect on %RM]

**Figure 5-23**: The effect of temperature on the power output signal obtained by the serial ramp of the methanol vapour pressure for amorphous indomethacin. Asterisks indicate the position of %cRMg, whereas arrows indicate the position of %cRMcry.

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>%cRMg</th>
<th>%cRMcry</th>
</tr>
</thead>
<tbody>
<tr>
<td>25</td>
<td>16.96</td>
<td>34.36</td>
</tr>
<tr>
<td>35</td>
<td>12.54</td>
<td>30.26</td>
</tr>
<tr>
<td>40</td>
<td>5.68</td>
<td>23.50</td>
</tr>
</tbody>
</table>

**Table 5-2**: Critical relative vapour pressure values of methanol (%RM) for amorphous indomethacin at different temperatures, %cRMg was %RM corresponding to the peak of the take off region (shown by the asterisk in Figure 5-23) and %cRMcry corresponding to the onset of the crystallisation.

When isothermal temperature for the serial ramp of methanol vapour pressure was increased a clear shift in critical vapour pressure values for amorphous indomethacin was observed (Figure 5-23). The critical values of methanol vapour pressure obtained from Figure 5-23 are listed in Table 5-2. The value of critical vapour pressure of methanol required to lower Tg of dry amorphous indomethacin to experimental temperature decreased from 16.96% (at 25°C) to 12.54% (at 35°C). Similarly the value of critical vapour pressure of methanol required to induce crystallisation in amorphous indomethacin decreased from 34.36% (at 25°C) to 30.26% (at 35°C). At
40°C, though it was difficult to estimate %cRMg, though the onset of crystallisation could be clearly observed at 23.5% (%cRMcry).

![Figure 5-24](image)

**Figure 5-24**: The values of critical %RM obtained from Table 5-2 plotted as a direct function of 1/T (K⁻¹).

In order to understand the kind of relationship between critical %RM values (c%RMg and %cRMcry) and temperature, the values of %cRM were plotted directly against 1/T. A straight line relationship was observed for both %cRMg and %cRMcry versus 1/T, which could be assessed from R² values (Figure 5-24). The equation of the straight line could characterise the individual relationship. The extrapolation of straight line to 0%RM should correlate to a value of temperature where the corresponding transitions are observed under dry conditions. This means, extrapolation of %cRMg line to 0%RM should yield the dry Tg, whereas that of %cRMcry line should be the spontaneous crystallisation temperature of dry amorphous indomethacin. The extrapolation of %cRMg and %cRMcry line produced temperature values of 50.94 and 84.27°C respectively (Table 5-3) which correspond to Tg (45-50°C) and Tcry crystallisation temperature of amorphous indomethacin respectively (as seen from Figure 3-1).

Lowering of the isothermal temperature should increase critical %RM values required to induce glass transition (%cRMg) and crystallisation (%cRMcry) in amorphous indomethacin. Since molecular mobility in the amorphous state also depends on temperature, it follows that the value of %cRM is an indication of molecular mobility in the amorphous state. The higher value of %cRM required to induce transitions in the amorphous solid with the reduction in isothermal temperature also follows a decrease
in molecular mobility. The maximum possible value of %cRM required to induce transitions in amorphous solid could indicate the temperature of least possible molecular mobility (Kauzmann temperature\textsuperscript{13}, Tk). The extrapolation of %cRM\textsubscript{g} and %cRM\textsubscript{cry} lines to 100%RM would correlate to temperature values of -56.16 and -45.11°C respectively (Table 5-3). The values of Kauzmann temperature reported in literature using different techniques are shown in Table 5-4.

![Graph showing exponential function of 1/T (K⁻¹)](y = 2178.2x - 3.7458, y = 6182x - 17.823)

**Figure 5-25:** The values of critical %RM obtained from Table 5-2 plotted as an exponential function of 1/T (K⁻¹).

<table>
<thead>
<tr>
<th>Extrapolated Temperature (°C)</th>
<th>0%RM\textsubscript{g}</th>
<th>0%RM\textsubscript{cry}</th>
<th>100%RM\textsubscript{g}</th>
<th>100%RM\textsubscript{cry}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Direct Relation</td>
<td>50.94</td>
<td>84.27</td>
<td>-56.16</td>
<td>-45.11</td>
</tr>
<tr>
<td>Arrhenius Relation</td>
<td>-</td>
<td>-</td>
<td>2.63</td>
<td>-12.17</td>
</tr>
</tbody>
</table>

**Table 5-3:** The values of temperature obtained by extrapolation of %cRM to 0 and 100%RM using direct and exponential relationship shown in Figure 5-24 and Figure 5-25.

\textsuperscript{13} Kauzmann temperature Tk, is believed to be a temperature of zero molecular mobility in amorphous solid. Storage and handling of amorphous solid at and below Tk should avoid any chances of crystallisation.
<table>
<thead>
<tr>
<th>Method</th>
<th>Kauzmann Temperature (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enthalpy relaxation</td>
<td>-6</td>
</tr>
<tr>
<td>Viscosity</td>
<td>-17</td>
</tr>
<tr>
<td>Enthalpy data</td>
<td>-79</td>
</tr>
<tr>
<td>Entropy data</td>
<td>-33</td>
</tr>
</tbody>
</table>

**Table 5-4:** The reported values of Kauzmann temperature obtained using various method for amorphous indomethacin from (Shamblin et al., 1999).

The plot of natural logarithm of critical %RM for amorphous indomethacin at different temperatures as a function of $1/T$ (Arrhenious relationship) is shown in Figure 5-25. The temperature values obtained by extrapolation of Arrhenious dependence of %cRM over $1/T$ are displayed in Figure 5-3. Tk value obtained using Arrhenious relation was higher as compared to the value obtained using direct relation between %cRM and temperature (Table 5-3). The values of Tk published in the literature using different approaches are shown in Table 5-4.
5.4.5 A comparison of interaction of the amorphous indomethacin with different alcohol vapours

![Graph showing comparison of interaction of amorphous indomethacin with different alcohol vapours](image)

**Figure 5-26:** A comparison of power output signal obtained by a serial ramp of vapour pressure of different alcohol vapour for amorphous indomethacin at 25°C.

<table>
<thead>
<tr>
<th></th>
<th>Methanol</th>
<th>Ethanol</th>
<th>n-Propanol</th>
<th>Water</th>
</tr>
</thead>
<tbody>
<tr>
<td>$T_g$ (K)</td>
<td>102-110</td>
<td>97-100</td>
<td>98-109</td>
<td>135-136</td>
</tr>
<tr>
<td>$T_m$ (K)</td>
<td>175</td>
<td>155.7</td>
<td>146.6</td>
<td>273</td>
</tr>
<tr>
<td>$T_b$ (K)</td>
<td>337.6</td>
<td>351.5</td>
<td>370.1</td>
<td>373</td>
</tr>
<tr>
<td>$T_b/T_m$</td>
<td>1.93</td>
<td>2.26</td>
<td>2.52</td>
<td>1.37</td>
</tr>
<tr>
<td>$T_m/T_g$</td>
<td>1.79</td>
<td>1.67</td>
<td>1.48</td>
<td>1.85</td>
</tr>
<tr>
<td>Polarity Index</td>
<td>6.6</td>
<td>5.2</td>
<td>4.3</td>
<td>9</td>
</tr>
<tr>
<td>$p^o$ (torr) at 25°C</td>
<td>126.39</td>
<td>58.7</td>
<td>21.76</td>
<td>23.77</td>
</tr>
<tr>
<td>$\Delta H_{vap}$ (kJ/mole)</td>
<td>--</td>
<td>39.3</td>
<td>--</td>
<td>59.0</td>
</tr>
<tr>
<td>Density (kg/m³)</td>
<td>0.79</td>
<td>0.785</td>
<td>0.8</td>
<td>0.997</td>
</tr>
</tbody>
</table>

**Table 5-5:** Various physical parameters of different solvents may be of relevance in the nature of the interaction with the amorphous indomethacin. Where $T_g$, $T_m$ and $T_b$ are the temperatures corresponding to glass transition, melting and boiling transitions respectively, source (Angell et al., 1978).

A comparison of heat flow signal obtained by a serial ramp of alcohol vapour pressure for amorphous indomethacin is performed in Figure 5-26. Although glass transition and crystallisation events could be clearly seen over the time scale of experiment for all the alcohols, there was a difference for the amount of vapour pressure required to induce these transitions. A significant difference was observed in the vapour pressure of these alcohols required to induce crystallisation in amorphous indomethacin at 25°C. The properties of different alcohols which could be of relevance are listed in Table 5-5.
5.5 **Summary of microcalorimetric studies on interaction of solvent vapour with amorphous solid**

- Amorphous lactose exhibited clear transitions in power output signal obtained by a serial ramp of RH. These were mainly glass transition, collapse and crystallisation of amorphous solid. The range of RH over which transitions were observed in power output signal (obtained by TAM) matched with the range of RH over which a changes in observed in water uptake behaviour of amorphous lactose (obtained by DVS). The value of RH at which changes were observed in power output signal could be correlated to critical RH (%cRH) for amorphous lactose.

- In the case of amorphous indomethacin only the glass transition could be observed by using a serial RH ramp at 25°C, as compared to glass transition and crystallisation observed with amorphous lactose.

- The transitions in amorphous indomethacin viz. glass transition and crystallisation could be followed by using a serial ramp of aliphatic alcohol vapour such as methanol, ethanol and n-propanol.

- When amorphous indomethacin was studied at different isothermal temperatures using a serial ramp of methanol vapour pressure, the value of %cRM for glass transition and crystallisation decreased as the temperature was increased. In %cRMg (to induce glass transition) and Ln %cRMcry (to induce crystallisation) exhibited a linear relationship against 1/T. Kauzmann temperature (Tk, temperature of zero molecular mobility) calculated for amorphous indomethacin by extrapolating Ln %cRMg and Ln%cRMcry against 1/T line correlated to 2.63 and -12.17°C respectively. Tk value calculated using the approach described in the work was in agreement with the Tk values reported in previous studies.
Chapter 6  Characterisation of amorphous solid using inverse phase gas chromatography
6.0 Characterisation of amorphous solid using Inverse phase gas chromatography (IGC)

6.1 Background, IGC for the characterisation of amorphous materials

IGC involves characterisation of the solid packed in a column as the stationary phase. Standard gaseous probes with known properties are then eluted through the column using a non-interacting carrier gas (e.g. Helium). The solid phase is then characterised based on its interaction with the gaseous probes. Since interactions between the solid stationary phase of the packed column and the probe molecules are primarily at the surface of the solid, IGC mainly enlightens surface properties of the solid. The characterisation of solid surfaces using IGC involves assessment of the components of surface energy (e.g. the dispersive or non-polar component and the polar component i.e. acidic and basic nature) (Hamieh et al., 2002; Newell et al., 2001b). The technique of surface energy measurement by IGC involves (Newell et al., 2001b) injection of a series of non-polar (alkane) and polar probes. The surface energy value measured represents the average of all energetic interactions between solid surface and gaseous probes. The dispersive component of the surface energy ($\gamma_d$) measured by IGC was successfully used to differentiate between amorphous state ($\gamma_d$ value of $37.1\pm2.3$ mJ m$^{-2}$) and crystalline state ($\gamma_d$ value of $31.2\pm1.1$ mJ m$^{-2}$) of lactose (Newell et al., 2001b). IGC has also demonstrated that the milling induced disorders were mainly localized at the surface of particles (Newell et al., 2001a). Although IGC proved useful to differentiate between amorphous and crystalline states of lactose, it is difficult to assess the chemical nature of the surface from the surface energy value. In an interesting study it was observed that many compounds with very different chemical nature exhibited a very similar dispersive energy component ($\gamma_d$) (Planinsek and Buckton, 2003).

IGC was also used to follow the transformation from amorphous to crystalline lactose induced by water vapour (Newell et al., 2001b). In this study it was observed that the surface energy ($\gamma_d$) of amorphous lactose (milled) decreased linearly to a value similar to crystalline lactose monohydrate over 0-40%RH. The change in surface energy was only reversible up till 20%RH.
Figure 6-1: The effect of temperature on dispersive surface energy for PMMA (from (Hamieh and Schultz, 2002). The variation of surface energy as a function of temperature could be used to study the transitions of solid at the surface. The effect of temperature on the dispersive component of surface energy studied for PMMA (poly-methyl-methacrylate) (Hamieh and Schultz, 2002) exhibited three clear maxima (Figure 6-1). The values of temperature corresponding to each maximum were correlated to a transition in PMMA as, β-relaxation (T1), glass transition Tg (T2) and liquid-liquid transition or order-disorder transition (T3). Although it is interesting to know the reasons for the maxima in dispersive surface energy at these transitions, of great interest is that IGC demonstrated its use in studying the polymer at different temperatures.

The similar transitions in amorphous PMMA were also observed by studying the retention behaviour of a single probe gas at various temperatures and then plotting the relationship between RT lnVn against 1/T (Figure 6-2) (Hamieh and Schultz, 2002). Generally a plot of RT lnVn against 1/T for crystalline substances is observed as a straight line with the slope as a measure of enthalpy change due to the adsorption process (ΔHads). In the case of amorphous PMMA clear breaks in straight line relationship between RT lnVn against 1/T were observed at some temperature values. These temperatures were correlated to transitions in amorphous PMMA as explained previously. The changes in retention behaviour were attributed to changes in nature of interaction between the polymer surface and the probe molecules, hence to changes in physical nature of the polymer. Since the glass transition is
accompanied with a change in physical nature of the substance (glassy to rubbery), changes in retention behaviour of the probe gas could be anticipated at/around Tg.

**Figure 6-2:** A variation of RT InVn of PMMA as a function 1/T using nonane as a probe. (From, (Hamieh and Schultz, 2002).

The glass transition temperature (Tg) obtained using the above mentioned approach has been used widely for polymers and carbohydrates with the estimated Tg values comparable to those obtained using other techniques such as DSC (Delarue and Giampaoli, 2000; Surana et al., 2003).

The sorbed water can act as a plasticizer, the amount of sorbed water depends on RH, hence the measured Tg value could be influenced if RH is not controlled. The unique advantage of IGC is its absolute control over RH of the carrier gas and hence RH of the atmosphere in which glass transition is estimated.

IGC could also be an invaluable tool to study glass transition of strong glasses (Angel's classification of glasses) (section 1.6.1). Since the strong glasses (e.g. many polymers) exhibit weak calorimetric transition (a small change in specific heat at glass transition) hence a DSC heating scan could be devoid of any observable Tg.

The different approaches adapted in this study to determine the Tg of the amorphous state involved estimation of retention volume of gaseous probe either as a function of temperature or humidity. As a first approach, humidity was kept constant and then temperature was increased, whereas in the second approach temperature was held constant and humidity was increased.
6.2 Estimation of Tg for amorphous indomethacin using IGC

Figure 6-3: Retention volume (Vn) of decane plotted as ln(Vn/T) against inverse of temperature (1/T) for amorphous indomethacin. The value of temperature corresponding to intercept of two lines was correlated to T_g of amorphous indomethacin.

Amorphous indomethacin (<350μm) was packed in a glass column and temperature was increased in steps of 5°C. After about 45 min. of equilibration at each temperature the elution of decane was performed using dry helium as a carrier gas. The retention volume (V_n) of decane obtained at each temperature step was plotted as ln(V_n) against 1/T (Figure 6-3). It could be well anticipated that with increase in temperature the amorphous state would undergo a glass transition (transition from glassy state to rubbery state). The retention properties of solid amorphous surface should change as glassy state transits into a more rubbery or a liquid like state. The change in retention behaviour of amorphous indomethacin was observed as a break in the plot of ln(Vn/T) against 1/T and dry T_g was calculated as shown in Figure 6-3.

The use of IGC in studying T_g by the above mentioned method has been reported mainly for polymers (Hamieh and Schultz, 2002; Ourdani and Amrani, 2002) and for carbohydrates (Delarue and Giampaoli, 2000). This technique was particularly useful in studying the miscibility of two or more polymers. The miscibility of polystyrene with poly(ethyl methacrylate) was observed as single composition dependent T_g (Ourdani

14 The equilibration time was fixed to 45 min for each step using the iGC software.
and Amrani, 2002). Using IGC it was also possible to show that carbohydrate matrixes retain the aroma (flavours) more strongly on a rubbery state as compared to glassy state (Delarue and Giampaoli, 2000). This study was performed using aroma compounds as probe molecules and carbohydrates as stationary phase.
6.3 A comparison of various techniques for the measurement of Tg

![Graph showing Tg values for various techniques](image)

**Figure 6-4:** The glass transition temperature (Tg) of amorphous indomethacin observed with StepScan-DSC, DSC, Hyper-DSC (onsets) and IGC showing the dependence of method used in estimation of Tg, (n=3).

There are several techniques which are reported for measurement of glass transition phenomena, e.g. thermal calorimetric techniques (DSC, High speed DSC), thermal mechanical techniques (DMA), spectroscopic techniques (FTIR) etc.

In this work glass transition was characterised by several calorimetric techniques including conventional DSC, high speed DSC, StepScan DSC and the results were compared with IGC measurements (Figure 6-4). It is very clear that although all the techniques targeted towards estimation of same physical transition (viz. glass transition) different results could be obtained (the value of Tg depended on the measurement technique).

The heating rate dependence of Tg is well known and could be a major contributing factor in differences in Tg estimated using calorimetric techniques. Most commonly the heating rate of 10°C/min is used in estimation of Tg but a comparison between IGC and conventional DSC indicated difference of about 9-10°C in Tg measured for amorphous indomethacin. In a technical viewpoint, IGC analysis is inherently based on absolute dry conditions for the measurement of Tg since dry helium was employed as a carrier gas. This is coupled with initial drying step which removes any adsorbed
moisture from the solid sample. On the contrary there was as such no control over the sample handling and analysis during DSC characterisation. It is also very difficult to ensure complete dryness of the solid sample prior to DSC analysis. The importance of these contributing factors viz. the dry measurement conditions and the dryness of solid sample could be easily reflected in the different values of Tg estimated from IGC and DSC.

<table>
<thead>
<tr>
<th>Tg (°C)</th>
<th>Heating rate (°C/min.)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>42</td>
<td>10</td>
<td>(Matsumoto and Zografi, 1999)</td>
</tr>
<tr>
<td>44.7</td>
<td>20</td>
<td>(Tong and Zografi, 1999)</td>
</tr>
<tr>
<td>-50</td>
<td>20</td>
<td>(Yoshioka et al., 1994)</td>
</tr>
<tr>
<td>50</td>
<td>20</td>
<td>(Yoshioka et al., 1995)</td>
</tr>
<tr>
<td>47</td>
<td>20</td>
<td>(Hancock et al., 1995)</td>
</tr>
<tr>
<td>44.7±1.3</td>
<td>20</td>
<td>(Tong and Zografi, 2001)</td>
</tr>
<tr>
<td>44.1</td>
<td>1 (MDSC)</td>
<td></td>
</tr>
</tbody>
</table>

Table 6-1: The values of Tg reported in literature (Tg onset) showing variation from 42 to 50°C for Tg of amorphous indomethacin.

It was also interesting to note that different Tg values were reported in different studies for amorphous indomethacin (Table 6-1). The differences in Tg values shown in Table 6-1 could possibly argue the importance of a control over RH of Tg measuring conditions.
6.4 Estimation of glass transition temperature under controlled conditions of temperature and RH using IGC

Instead of using dry helium as a carrier gas, a known amount of water vapour could also be introduced along with helium to study the plasticisation effect of water vapour. Most commonly, the plasticisation of the amorphous state by sorbed water was studied by storing the amorphous solid in a desiccator equilibrated with a suitable salt solution; the plasticised solid was then taken out and characterised using DSC. Although this method could give a rapid estimate of the plasticisation effect it is also accompanied with a few technical limitations.

- In the case of hydrophobic compounds the distribution of the sorbed water would be uneven this could mean that the plasticisation effect of sorbed water would be different over the particle. Since the vapour sorption proceeds from the surface of a particle (the point of contact between water vapour and solid) there could be a concentration gradient of water with more water at the surface and less in the bulk.

- The heating scan employed in DSC measurement could easily lead to redistribution of water in the bulk of the particle and hence characterisation of the amorphous state with respect to Tg could be ambiguous.

In the next part of the thesis a novel use of IGC in determining the effect of plasticisation of water has been studied using amorphous indometacin (hydrophobic model) and amorphous lactose (hydrophilic model).
6.4.1 Studies on plasticisation of amorphous indomethacin by sorbed water

Amorphous indomethacin was packed in a glass column and then dried under a flow of dry helium gas whilst maintaining isothermally at 30°C using the column oven. The RH of the carrier gas was then increased in a stepwise manner. At each RH step the column was equilibrated for about 45 min after which decane was eluted as a probe gas. The elutions were performed whilst maintaining the RH of the carrier gas. The retention volume for the decane peak was calculated as $V_{\text{max}}$ (using the time corresponding to the maximum peak height) and $V_{\text{com}}$ (using the time corresponding to the centre of mass of the peak) by using the IGC analysis software.

![Graph showing retention volumes for decane on amorphous indomethacin as a function of relative humidity at 30°C. Arrow shows the designated critical RH](image)

**Figure 6-5:** Retention volumes for decane on amorphous indomethacin as a function of relative humidity at 30°C. Arrow shows the designated critical RH

The plot obtained for retention volume against %RH is shown in Figure 6-5. It could be seen from Figure 6-5 that initially, till about 60%RH, the retention volume of decane decreased with the rise in RH. This could be due to occupation of high energy sites on amorphous solid by adsorbed water molecules. Below 60%RH, the change in two retention volumes ($V_{\text{max}}$ and $V_{\text{com}}$) matched each other or both decreased in a similar manner. Above 65%RH both values started increasing but the increase in $V_{\text{com}}$ was more as compared to $V_{\text{max}}$. Firstly the increase in retention volume meant that even if solid surface might be assumed to be getting more hydrophilic (due to increase in RH) its interaction with the hydrophobic probe (decane) was getting stronger. The more rapid increase in $V_{\text{com}}$ values (beyond 65%RH) as compared to $V_{\text{max}}$ values indicated that the decane peak started tailing increasingly. The
increased interactions (from the rise in retention volume) and the increased tailing (sharp rise in Vcom values) meant that the decane was retained more in the amorphous solid above 65%RH. This is due to transformation of the amorphous solid from a glassy state (solid like) to a rubbery state (liquid like, less viscous) beyond 65%RH. The rubbery state would pose more free access to decane molecules as compared to glassy state, hence the interaction between amorphous indomethacin (above 65%RH) and decane molecules would not only be limited to surface adsorption but might also include partitioning of decane into the rubbery state.

![Graph showing retention volumes for decane on crystalline lactose monohydrate (Foremost) as a function of relative humidity at 30°C.](image)

**Figure 6-6:** Retention volumes for decane on crystalline lactose monohydrate (Foremost) as a function of relative humidity at 30°C.

In another experiment crystalline solid was packed as a stationary phase. The retention volume of decane was measured in a similar way at increasing steps of RH. For crystalline lactose monohydrate (Figure 6-6) and crystalline indomethacin (Figure 6-7) the retention volume did not increase at high RH values (as seen for amorphous indomethacin). These observations supported the fact that increased interaction of decane with amorphous indomethacin (Figure 6-5) was due to plasticisation effect of sorbed water which lowered the Tg of dry amorphous solid to the experimental temperature (30°C). The value of RH at which the phase transition was initiated could be regarded as a critical RH (%cRHg) at 30°C for amorphous indomethacin.

The %cRHg (RH required to lower the dry glass transition to the experimental temperature) for amorphous indomethacin obtained at different temperatures were plotted as a function of inverse temperature (Figure 6-8). With the rise in temperature
there was a linear decrease in %cRHg. It could be anticipated that the extrapolation of straight line to 0%RH should correspond to a temperature at which dry Tg of amorphous indomethacin could be observed. The extrapolation of the straight line to 0%RH gave a temperature value of 48.6°C which corresponded very well with dry glass transition of amorphous indomethacin obtained in this study and reported before (Table 6-1).

![Graph](image)

**Figure 6-7:** Retention volumes for decane on crystalline indomethacin as a function of relative humidity at 30°C.

Based on the Gordon Taylor equation (Gordon and Taylor, 1952) the amount of water content needed to lower Tg of indomethacin to 35°C was 2.44 %w/w (using 48.6°C as Tg of amorphous indomethacin by IGC) and 1.8%w/w (using 44.7°C as Tg of amorphous indomethacin from literature) (Tong and Zografi, 1999; Tong and Zografi, 2001). IGC results showed that %cRHg at 35°C was 50%. DVS water sorption results showed that the mass gain for amorphous indomethacin at 35°C and 50%RH was just below 1% w/w (Figure 4-12). Assuming that the mass of water sorbed in the IGC experiment was similar to that sorbed in DVS\(^\text{15}\), then the amount of water actually sorbed to lower the Tg to of the surface of the powder to 35°C (1%), was lower than that calculated using Gordon Taylor equation (2.44 and 1.8%w/w). It followed that the concentration of sorbed water at the surface was much higher than at the surface.

\(^{15}\) This was a perfectly reasonable assumption as the rate of sorption was clearly limited by the solid itself and not by the rate of supply of water vapour. The rate of supply of water vapour was rapid in both the DVS and IGC and both the DVS and IGC experiments show pseudo equilibration i.e. levelling of mass gain and the IGC shows a flat response with the thermal conductivity meter.
bulk (i.e. amount of water at the surface was high enough to lower Tg to experimental temperature, even though the sorbed mass in the entire sample was too low to reduce Tg of entire sample mass to T). These results would be consistent with a concentration gradient of water through the hydrophobic mass, with surface being plasticised to a greater extent than bulk of the sample.

Figure 6-8: Plot of %cRHg (after which there was a deviation between Vcom and Vmax) as a function of 1/T (K⁻¹) showing straight line relationship (line equation y = 338238x - 1051.8 and R²=0.9976; value of extrapolated dry Tg = 48.6°C).

Previous studies reported a complex crystallisation behaviour of amorphous indomethacin due to an exposure to high RH (Andronis et al., 1997). The authors also noted that the crystallisation was mainly initiated at the surface of particles. The surface crystallisation of amorphous indomethacin was correlated to higher water content and molecular mobility at the surface relative to bulk of the particle.

In this study IGC has been shown to be a useful tool to distinguish surface plasticisation from bulk plasticisation.
6.4.2 Studies on plasticisation of amorphous lactose by sorbed water

In the previous section the plasticisation effect of sorbed water was studied using a hydrophobic model (amorphous indometacin) where the overall effect was limited to the surface of the particle. This may not be the case with hydrophilic substances where water distribution could be more rapid and uniform throughout the bulk of the particle. In this study transitions in amorphous lactose were followed as a function of RH using Inverse gas chromatography (IGC).

![Graph](attachment:graph.png)

**Figure 6-9:** Retention volume calculated (Vcom and Vmax) and pressure drop across amorphous lactose column at 30°C, initially 0%RH and sequentially increasing RH. Arrows indicating the transitions in amorphous lactose, first transition (T1), glass transition (T2) and collapse (T3).

It could be seen from Figure 6-9 that, unlike amorphous indometacin (Figure 6-5) there was no place at which a break between Vcom and Vmax was observed. For amorphous indometacin the point at which Vcom and Vmax deviated (i.e. the point at which there was an increase in tailing of retention peak) was taken to be the point at which the surface had been plasticised such that Tg = T, (Buckton et al., 2004). The rationale for this study was the correlation of the change in ability of the sample to absorb decane to a transition in amorphous indometacin. In the case of lactose, Vmax and Vcom values were similar and did not deviate, which would indicate that there was little tendency for decane to absorb into amorphous lactose. There is further work required to see if other probes could be selected, which would show changes in...
absorption and hence a deviation between Vmax and Vcom for lactose. Despite the fact that Vmax and Vcom did not deviate from each other as RH changed, it was clear (Figure 6-9) that the retention volume changed as a function of humidity. Also plotted in Figure 6-9 was pressure drop across the column, and it was clear that there were two regions in which this changed, one after 10% RH and another after 35%RH. Each change was taken as a transition point for amorphous lactose and studied at different temperatures.

6.4.2.1 The low RH transition region (ca 10% RH)

![Retention volume Vmax vs. RH](image)

**Figure 6-10:** The plot of retention volume (Vmax) of decane against RH, arrows show the first transition in amorphous lactose.

The low RH region (in Figure 6-9) was explored further by studying the retention behaviour of decane as a function of temperature and RH. It could be seen (Figure 6-10) that retention behaviour showed a clear break at the following conditions, 25°C/18%RH; 30°C/12%RH; 35°C/9% RH and 40°C/8% RH. These transitions exactly matched the change in pressure drop in the column (Figure 6-11), demonstrating that the sample is altering its geometry at a defined point, which alters the retention volume of decane (Vmax) and pressure drop in the column; in either case (i.e. either change in Vmax or pressure drop) it was clear that there was a critical point.
The water uptake for amorphous lactose at each temperature was discussed previously (section 4.1). It could be seen that from 8-18%RH the mass change started deviating between each isotherm. At 25°C/17.2%RH about 2.5% water could be absorbed by amorphous lactose which was equal to monolayer coverage (from BET equation). The Tg of amorphous lactose containing 2.5%w/w of sorbed water was equal to 74.3°C (calculated using Gordon and Taylor equation); for 2% water content the calculated Tg was 81°C and for 3% it was 67°C. This equated to Tg being approximately 40°C above the experimental temperature at the point of this transition, this was in keeping with the proximity to Tg below which there is no, and above which there is significant molecular mobility (Hancock and Zografi, 1997).

It is likely, therefore, that the IGC method is revealing a transition that equated to the onset of significant molecular mobility in amorphous material.
6.4.2.2 The second transition region (30-40% RH)

The retention volume of decane decreased with increase in RH for amorphous lactose at 30°C (Figure 6-9). The decrease in retention volume could be anticipated with increased hydrophilic character of amorphous lactose but the gradual increase was accompanied with a break (sudden or more rapid decrease) at 30-35%RH. The break in the retention volume line at 30-35%RH was followed by a sharp decline in pressure drop values at 35-40%RH. The collapse of amorphous lactose in the column was evident from the fall in pressure drop values, as the original column offered more resistance to the flow of carrier gas than the collapsed column (from 35-40%, Figure 6-9). Since the collapse in amorphous state follows glass transition it was likely that the break in retention volume line at 30-35%RH could be due to the transition from the glassy to rubbery state.

**Figure 6-12:** A comparison of glass transition and collapse seen using DVS and IGC at 25°C for amorphous lactose. Glass transition from change in retention volume (RH1), collapse from pressure drop (RH2), collapse from reduced water uptake by DVS (RH3), value predicted using Gordon Taylor equation 37%RH.

In Figure 6-12, IGC results were compared directly with water uptake results for amorphous lactose at 25°C obtained by DVS. It was discussed in section 4.1.1.2 that by using Gordon Taylor equation the calculated RH required for just sufficient water sorption to lower the dry glass transition temperature to experimental temperature
was equal to 37%RH. DVS weight change data exhibited the maximum step change at 40%RH at 25°C. The deviation in retention volume (Figure 6-12) is in keeping with the point where rate and extent of water sorption are seen to change. Hence, the break point in retention volume is a very clear indication of the sample passing through its glass transition.

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Glass transition (%cRHg)</th>
<th>Collapse (%RH)</th>
</tr>
</thead>
<tbody>
<tr>
<td>25</td>
<td>34-38</td>
<td>38-42</td>
</tr>
<tr>
<td>30</td>
<td>26-30</td>
<td>30-34</td>
</tr>
<tr>
<td>35</td>
<td>18-21</td>
<td>21-24</td>
</tr>
<tr>
<td>40</td>
<td>18-21</td>
<td>21-24</td>
</tr>
</tbody>
</table>

**Table 6-2**: The combination of temperature and %RH values for glass transition and collapse in amorphous lactose obtained by IGC.

The RH region in which there was a break in retention volume line (glass transition) and pressure drop (collapse), are shown in Table 6-2 for each experimental temperature. It could be seen that %RH required to lower the glass transition to experimental temperature decreased with increase in temperature. A similar effect was also observed for the collapse (%RH). It has been shown that IGC can identify Tg of amorphous materials by following retention of a single probe and that this is seen to correlate to the region where rate and extent of water sorption to amorphous material changes. IGC in combination with moisture sorption data has proved powerful in showing these four significant features (initial mobility, Tg, collapse, crystallisation) and this is seen as a valuable advance in the study of amorphous samples.
6.5 Estimation of zero mobility temperature (Tk)

In the previous sections of the thesis a number of techniques to characterise the amorphous state of different substances have been described. The amorphous state was characterised with respect to the critical relative vapour pressure of solvent (%cRS) required to induce a spontaneous transition (e.g. mobility onset, glass transition and crystallisation) at a particular temperature. The dependence of %cRS value on temperature indicated a link between molecular mobility and the estimated value of %cRS. This section deals with the estimation of zero mobility temperature (Tk) by extrapolating the relationship observed between various %cRS values and temperature.

6.5.1 Estimation of zero mobility temperature (Tk) for amorphous lactose

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Mobility onset (IGC)</th>
<th>Glass transition (%cRHg)</th>
<th>Crystallisation (%cRHcry)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>IGC</td>
<td>DVS</td>
</tr>
<tr>
<td>25</td>
<td>19.5</td>
<td>40</td>
<td>37</td>
</tr>
<tr>
<td>27</td>
<td>16.5</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>30</td>
<td>13.5</td>
<td>31.5</td>
<td>34.5</td>
</tr>
<tr>
<td>35</td>
<td>10.5</td>
<td>25.5</td>
<td>31.5</td>
</tr>
<tr>
<td>40</td>
<td>8.5</td>
<td>20.5</td>
<td>28.5</td>
</tr>
<tr>
<td>45</td>
<td>--</td>
<td>--</td>
<td>26</td>
</tr>
<tr>
<td>50</td>
<td>--</td>
<td>--</td>
<td>25</td>
</tr>
<tr>
<td>55</td>
<td>--</td>
<td>--</td>
<td>20.5</td>
</tr>
</tbody>
</table>

Table 6-3: The values of critical RH (%cRH) measured using different approaches as a function of temperature for amorphous lactose.

A list of different critical %RH values obtained for amorphous lactose using DVS and IGC are shown in Table 6-3. A clear dependence of temperature was observed for the estimated value of %cRH. As a next step dependence of %cRH on temperature was studied by plotting %cRH against 1/T using a direct relationship and exponential relationship. The equations of the straight lines obtained from the best fit and corresponding R² values are shown in Table 6-4; whereas equations of the straight lines, obtained by plotting natural logarithm of %cRH and 1/T are shown in Table 6-5. Extrapolation of mobility onset line to 0%RH should correspond to a value of temperature below which amorphous state would lack any molecular mobility. The temperature obtained using this method was 51.78°C, which was in agreement with the temperature at which no aging was observed when studied using enthalpy relaxation (Section 3.4).
Table 6-4: Equation of the straight line obtained by directly plotting %cRH against 1000/T and the values obtained by extrapolating line to 0 and 100%RH.

<table>
<thead>
<tr>
<th></th>
<th>Equation of straight line</th>
<th>R²</th>
<th>Extrapolation to %RH</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>0%RH</td>
</tr>
<tr>
<td>Mobility onset</td>
<td>( y = 66.096x - 203.51 )</td>
<td>0.95</td>
<td>51.78°C</td>
</tr>
<tr>
<td>(IGC)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glass transition</td>
<td>( y = 120.52x - 365.25 )</td>
<td>0.99</td>
<td>56.96°C</td>
</tr>
<tr>
<td>(IGC)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glass transition</td>
<td>( y = 53.13x - 141.08 )</td>
<td>0.99</td>
<td>103.57°C</td>
</tr>
<tr>
<td>(DVS)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Crystallisation</td>
<td>( y = 66.48x - 166.53 )</td>
<td>0.96</td>
<td>126°C</td>
</tr>
<tr>
<td>(DVS)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 6-5: Equation of the straight line obtained by plotting ln(%cRH) against 1000/T and the values obtained by extrapolating line to 100%RH.

<table>
<thead>
<tr>
<th></th>
<th>Equation of straight line</th>
<th>R²</th>
<th>Extrapolation to 100%RH</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mobility onset</td>
<td>( y = 5.0842x - 14.134 )</td>
<td>0.99</td>
<td>-1.7°C</td>
</tr>
<tr>
<td>(IGC)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glass transition</td>
<td>( y = 4.1358x - 10.93 )</td>
<td>0.99</td>
<td>6.48°C</td>
</tr>
<tr>
<td>(IGC)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glass transition</td>
<td>( y = 1.6283x - 1.844 )</td>
<td>0.99</td>
<td>-20.51°C</td>
</tr>
<tr>
<td>(DVS)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Crystallisation</td>
<td>( y = 1.444x - 0.7979 )</td>
<td>0.96</td>
<td>-6.114°C</td>
</tr>
<tr>
<td>(DVS)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Theoretically, extrapolation of the glass transition line to 0%RH should correlate to the dry Tg of amorphous lactose but the value obtained (56.96°C) was very much different than dry Tg of amorphous lactose (110°C). It was possible that amorphous lactose follows an exponential dependence of %cRHg on 1/T. Whereas the extrapolation to 0%RH correlated to the respective transition under dry conditions, the extrapolation to 100%RH should correlate to the value of temperature below which amorphous state has to be stored in order to avoid the transition at 100%RH. This meant that if the amorphous lactose has to be stored at 100%RH without any molecular mobility it has to be stored at below -55°C (using direct relationship) or -1.7°C (using exponential relationship).

Using this approach it was possible to estimate zero mobility temperatures for amorphous lactose as whilst taking into account the water content and %RH of storage conditions.
6.5.2 Estimation of zero mobility temperature (Tk) for amorphous indomethacin

<table>
<thead>
<tr>
<th>Equation of straight line</th>
<th>Direct relation</th>
<th>Exponential relation</th>
</tr>
</thead>
<tbody>
<tr>
<td>y = 292.31x - 899.61</td>
<td>y = 4.7364x - 11.466</td>
<td></td>
</tr>
<tr>
<td>R²</td>
<td>0.99</td>
<td>0.99</td>
</tr>
<tr>
<td>Extrapolation to</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0%RH</td>
<td>51.92°C</td>
<td>--</td>
</tr>
<tr>
<td>100%RH</td>
<td>19.42°C</td>
<td>21.71°C</td>
</tr>
</tbody>
</table>

Table 6-6: Equation of straight line obtained by plotting %cRH against 1000/T for direct relationship and ln(%cRH) against 1000/T for exponential relationship for amorphous indomethacin.

Equations of straight lines obtained by plotting direct relation and exponential relation are shown along with the extrapolation to 0%RH and 100%RH in Table 6-6. The important different between studies on amorphous state of indomethacin and lactose was the temperature range of IGC analysis. Whereas amorphous lactose was analyzed below Tk in the dry state of all analytical conditions amorphous indomethacin was analyzed above Tk or there existed some molecular mobility in amorphous state of indomethacin. Since the molecular mobility decreases with the decrease in temperature, the %cRHg would increase with the drop in temperature.

The extrapolated value of temperature corresponding the maximum RH (i.e. 100%RH from the exponential relation) would correlate to the least possible molecular mobility and hence to zero mobility temperature (Tk) in amorphous state of indomethacin.
Chapter 7  Characterisation of amorphous state using near infrared spectroscopy
7.0 Characterisation of amorphous state using near infrared spectroscopy

Various spectroscopic techniques such as FT-Infrared, Near infra-red, NMR, Raman and more recently Terahertz spectroscopy are being used in solid state characterisation of pharmaceuticals. The various applications for which these techniques are used include characterisation of polymorphic structures e.g. FT-IR (Rustichelli et al., 2000), Raman (Anwar et al., 1989); quantification of crystallinity in amorphous mixtures e.g. FT-Raman (Taylor and Zografi, 1998a) Near IR (Hogan and Buckton, 2001); extent of hydrogen bonding between different constituents e.g. FT-Raman (Taylor and Zografi, 1998b).

The spectroscopic techniques provide information about the position of peak absorption for a chemical bond (i.e. wave-number or frequency), along with peak distribution or a frequency range over which the peak is distributed. The peak absorption patterns obtained in the fingerprint region of IR spectra provide information about the presence of chemical functionalities, although this region is not very much affected by different lattice structures (e.g. polymorphism). Whereas IR spectra consist of fundamental vibrations of chemical bonds, Near-infrared (NIR) spectra consists of mainly overtones and the combinations of fundamental absorption modes.

NIR spectroscopy has been shown useful in distinction between amorphous and crystalline states of lactose (Buckton et al., 1998). FT-Raman and FT-IR spectroscopy has been shown useful in comparing the hydrogen bond patterns and strength between the crystalline and amorphous states of different dihydropyridine calcium channel blockers (Tang et al., 2002).

The hydrogen bonding studies using IR and Raman spectroscopy are based on estimation of peak position for X-H stretch. The free X-H stretch is characterised by a sharp peak, whereas on formation of hydrogen bond X-H···Y (where Y is the acceptor atom) the peak shifts to a lower wave-number and becomes much broader. This shift in wave-number is caused by the lengthening of X-H bond as a result of hydrogen bond formation. A stronger hydrogen bond will lengthen X-H more and produce a shift to a lower wave-number (Tang et al., 2002). This theory would not change while interpreting Near Infrared spectra instead of Infrared spectra.

In this work NIR spectroscopy is being attempted to understand the ongoing structural changes in an amorphous state (which lead to crystallisation).
NIR spectrum is normally plotted as absorbance against wavelength. This spectrum is the characteristic of chemical as well as the physical state of the sample. In this work mathematically treated NIR data as 2nd derivative SNV (standard normal variate) was used for the comparison between two NIR spectra. This mathematical treatment should remove any difference due to particle size but would result in downward displacement of the peaks. The peak absorption bands obtained from NIR spectra could be correlated to stretching vibrations of different chemical bonds. The most relevant absorption bands observed in the NIR range are due to C-H stretching, N-H stretching and C=O stretching vibrations along with their combinations and overtones.

\[ ^{16} \text{The peak obtained due to upward displacement of absorbance in the normal spectrum will correspond to the peak of downward displacement in 2^{nd} Derivative SNV due to the mathematical treatment.} \]
7.1 Solid state characterisation of amorphous indomethacin using near infrared spectroscopy

**Figure 7-1:** A comparison between NIR spectra of amorphous and crystalline (γ-form) indomethacin. The encircled part marks the difference in NIR absorption pattern of amorphous and crystalline state.

NIR spectra of the amorphous and crystalline state of indomethacin exhibited several differences (Figure 7-1) their possible correlation is summarized in Table 7-1.

The prominent differences between NIR spectra of amorphous and crystalline indomethacin were observed mainly in the region of C-H stretching absorption. Two distinct absorption bands were observed for crystalline indomethacin at 1346nm and 1384nm, whereas for amorphous state more than two absorption bands were observed in this wavelength range (1342-1416nm). The absorption in this wavelength range is generally observed due to 1st overtone of C-H combinations. Although multiple bands with differing intensities were observed for both crystalline and amorphous indomethacin in the region of 1610 to 1780nm their peak position remained the same. This wavelength region (1610-1780nm) mainly corresponds to the 1st overtone of C-H stretching vibration. Two distinct absorption bands were observed for crystalline indomethacin at 1988 and 2010nm, whereas for amorphous indomethacin a broad band was observed in this range with a peak at 2008nm and a shoulder at 1990nm. The absorption in this region of NIR corresponds to C=O 1st overtone and O-H combinations.
Table 7-1: A summary of peak NIR absorptions for amorphous and crystalline indomethacin.

Although the amorphous and crystalline indomethacin have the same chemical structure a multitude of differences were observed in their NIR spectra. These differences were mainly in the absorption region of C=O and O-H stretching bands and C-H overtones. The presence of two distinct C=O stretching bands at 1988 and 2010nm for the crystalline state could be due to separate absorption by two different carbonyl (ketonic and carboxyl carbonyl group) groups one of which exhibiting strong hydrogen bond (hence displaced to a higher wavelength i.e 2010nm). The single broad absorption band with a peak shifted to 1990nm in the amorphous state is possible if both carbonyl groups (ketonic and other carboxyl carbonyl) exhibited hydrogen bonding. The differences in C-H absorption bands in amorphous and crystalline state could indicate differing chemical environments in the two states of indomethacin.
7.2 Solid state characterisation of amorphous nifedipine using near infrared spectroscopy

Figure 7-2: A comparison of NIR spectrum of nifedipine in amorphous state with crystalline solid. Encircled part and arrows mark the differences in NIR absorption pattern of amorphous and crystalline state.

NIR spectrum of amorphous and crystalline nifedipine exhibited many differences, this included shifting and broadening of major peaks along with the presence of altogether new peaks for both states. A comparison between NIR spectra of amorphous and crystalline nifedipine is demonstrated in Figure 7-2. The major differences were observed in the region of N-H 1st overtone, C-H overtones and C=O stretch 2nd overtone (summarized in Table 7-1). In the crystalline state two small peaks were present at 1346 and 1390nm (in the region of 1st overtone of C-H combinations) as compared to three distinct peaks at 1346, 1370 and 1392nm in the amorphous state (additional peak at 1370 for amorphous solid). In the region of the 1st overtone of N-H stretch, a sharp peak at 1530 with a shoulder at 1510nm was observed for the crystalline state as compared to a broad peak at 1494nm for the amorphous state. In the wavelength range for 1st overtone of C-H stretch two distinct peaks at 1664 and 1700nm were observed for crystalline nifedipine; in the corresponding wavelength range a broad peak at 1664nm was observed for the amorphous state. The peak corresponding to 2nd overtone of C=O stretch at 2010nm for crystalline state was shifted to a lower wavelength (1998nm) for amorphous nifedipine.
Cry stalline n ifedipine | Amorphous n ifedipine | Possible correlation
---|---|---
Small peaks at 1346 and 1390nm | Distinct sharp peaks at 1346, 1370 and 1392nm | 1st overtone of C-H combinations
Sharp peak at 1530 with a shoulder at 1510nm | Broad peak at 1494nm | N-H 1st overtone
Distinct peaks at 1664 and 1700nm | Broad peak at 1664nm | 1st overtone of C-H
Peak at 2010nm | Peak at 1998nm | C=O stretch 2nd overtone

**Table 7-2**: A summary of peak NIR absorptions for amorphous and crystalline nifedipine.

The shifting of N-H and C=O stretching peak to a lower wavelength in the amorphous state as compared to crystalline state is an indication of lower hydrogen bonding in the amorphous as compared to the crystalline state. A similar finding was reported using FT-Raman and FT-IR spectroscopy for nifedipine (Tang et al., 2002).
7.3 Stability studies of amorphous indomethacin using NIR

![Graph showing 2nd Derivative SNV vs Wavelength (nm) for 0 h and 70 h storage at 25°C.]

Figure 7-3: The effect of storage at 25°C on NIR spectrum of amorphous indomethacin.

The effect of storage at 25°C for amorphous indomethacin studied using NIR is shown in Figure 7-3. It could be seen that NIR spectra of amorphous indomethacin remained the same over 70h of storage at 25°C. The molecular mobility studies using enthalpy recovery at 25°C showed the average relaxation time value to be 34 h for amorphous indomethacin (Section 3.4.3). Although the amorphous state exhibited molecular mobility at 25°C, NIR spectra of amorphous indomethacin remained unchanged over 70h. NIR spectrum of amorphous indomethacin stored over a longer duration at 25°C is shown in Figure 7-4. The NIR spectra of amorphous indomethacin changed gradually after storage at 5, 45 and 100 days. The major changes could be seen in the absorption region of 1st overtone of C-H combinations and combinations of C=O 1st overtone with O-H stretch (encircled parts in Figure 7-4). NIR spectra of amorphous indomethacin stored for 100 days at 25°C was closely similar to that of crystalline indomethacin. Although marked changes were observed in NIR spectra of amorphous indomethacin stored for 100 days at 25°C, little or no changes were observed for 100 days storage at 5°C

17 The peak at 1908nm for amorphous solid at 5°C is due to presence of moisture.
Figure 7-4: NIR spectra of amorphous indometacin taken at various time intervals after storage at 25°C (0%RH). The encircled part demonstrates the structural changes as the process of crystallisation proceeds in the amorphous state at 25°C (0%RH).

Figure 7-5: The effect of storage temperature on the rate of crystallisation as demonstrated by NIR spectra of amorphous indometacin stored at 5 and 25°C for 100 days. The encircled parts show the region where gross changes were observed.
7.4 Stability studies of amorphous nifedipine using NIR

![NIR Spectra Diagram](image)

**Figure 7-6:** The effect of storage temperature on NIR spectra of amorphous nifedipine.

Whereas amorphous indomethacin did not show any change in the NIR spectra for storage of 70h at 25°C (Section 7.3, Figure 7-3), amorphous nifedipine exhibited many changes within 22h of storage at 25 and 40°C. The prominent changes observed in NIR spectra for the amorphous nifedipine were in the wavelength region where N-H, C-H and C=O exhibited absorption bands (Figure 7-6). The peak at 1494nm due to N-H stretching for the initial state of amorphous nifedipine was shifted to 1520nm (for 22h at 25°C) and 1522nm (for 22h at 40°C) as compared to 1530nm in crystalline nifedipine. The broad peak at 1664nm (due to C-H stretch) in the initial state was gradually divided into two peaks at 25°C and at 40°C. The broad peak at 1998nm (due to C=O stretch) for initial amorphous state was narrowed after 22h at 25°C. Although peak positions remained the same for 22h of storage at 25 and 40°C, the peak intensity was higher (for all the peaks) at 40°C as compared to 25°C.

All these changes in the NIR spectra of amorphous nifedipine are clear indication of a gradual increase in the strength of hydrogen bonds. Although it is not necessary that the amorphous nifedipine would transform into a given polymorphic form, its spectra was evolving towards the form used as a standard for the crystalline state in this study.
Figure 7-7: The evolution of the NIR spectra of amorphous nifedipine at 25°C, encircled region shows the changes in the peak position.

Figure 7-8: The evolution of the NIR spectra of amorphous nifedipine at 40°C, encircled region shows the changes in the peak position.

The NIR spectra recorded over time intervals for amorphous nifedipine by keeping isothermally at 25 and 40°C are shown in Figure 7-7 and Figure 7-8 respectively. The
evolution process of N-H stretching peak at 1494nm and C-H stretching peak at 1664nm could be seen clearly. This process was accelerated at 40°C (Figure 7-8) as compared to 25°C (Figure 7-7).
7.5 Interpretation of NIR spectra to study physical transitions in amorphous state

Thermodynamically, aging of the amorphous state could be treated as reversible whereas crystallisation an irreversible transition. The estimate of enthalpy relaxation was used to study the process of aging in amorphous solids (Section 3.4). It was difficult to distinguish between the two stages (viz. aging and crystallisation) by using NIR spectroscopy. The changes in NIR spectra could be interpreted as physical transformations of the solid state.

![NIR Spectra of Amorphous Indomethacin](image)

**Figure 7-9:** The NIR spectra of amorphous indomethacin after 5 and 10 days of storage at 25°C, the dotted circle shows the part of spectra where changes were observed whereas the solid circle shows the part in which the spectra remained unchanged over 10 days.

In the case of amorphous indomethacin extended storage (5-10 days) at 25°C was accompanied with changes in mainly two regions of the NIR spectra. The changes were observed in the band position for the 1st overtone of C-H combinations (1340-1420nm) and C=O 1st overtone combined with the O-H stretch (1976-2026nm) (Figure 7-9). The NIR spectra of amorphous indomethacin recorded over 10 days of storage at 25°C showed changes only in the 1340-1420nm wavelength range whereas bands at 1976-2026nm range remained unchanged (Figure 7-9). It was only after 45 days of storage at 25°C the changes were observed in absorption bands at 1976-2026nm (Figure 7-4). Since the amorphous indomethacin exhibited molecular mobility at 25°C
(average relaxation time of 34h, Table 3.6), it is possible to correlate changes in C-H absorption band position in the 1340-1420nm range to the aging of the amorphous solid. It is only the changes in absorption bands of C=O and O-H which correlates to the process of crystallisation in amorphous indometacin. This is a reasonable conclusion since the process of crystallisation generally follows inter and/or intramolecular hydrogen bonding and in indometacin the only group which could be involved in hydrogen bonding is –COOH.

In the case of nifedipine it is the N-H group, which will be involved in the formation of hydrogen bonds hence the process of crystallisation would clearly accompany changes in the band position for the N-H stretch. The changes were observed in band position of the N-H stretch within a couple of hour's storage of amorphous nifedipine at 25°C (Figure 7-7) and 40°C (Figure 7-8). The enthalpy relaxation results indicated remarkable dependence of molecular mobility on storage temperature, since the average relaxation time changed from 0.85h at 35°C to 2098h at 25°C. The NIR studies indicated absence of any lag time between aging and crystallisation of amorphous nifedipine. This was in contrast to amorphous indometacin where crystallisation could be observed only after a few days of storage at 25°C.
Chapter 8  Future work and summary
8.0 Future work and summary

8.1 Estimation of enthalpy of solution as a function of temperature

The enthalpy change associated with the solubilization of any solid ($\Delta H$) consists of mainly two components, one due to the breaking of lattice structure of the solid ($\Delta H_{\text{lattice}}$) and second the formation of bonds with the liquid molecules ($\Delta H_{\text{solvation}}$).

\[
\Delta H = \Delta H_{\text{lattice}} + \Delta H_{\text{solvation}}
\]

It could be assumed that the second component (i.e. heat associated with formation of bonds between solid and liquid molecule) ($\Delta H_{\text{solvation}}$) would remain unaffected for different solid states of the same molecule (i.e. for amorphous and crystalline state). The difference observed in heat of solution values ($\Delta H$) between amorphous and crystalline states, as a function of temperature would correlate to the susceptibility of the amorphous state to the change in temperature. The value of temperature at which $\Delta H$ reaches zero could be correlated to the Kauzmann temperature (temperature of zero molecular mobility).

This approach is based on estimation of Gibbs free energy difference $\Delta G$, against temperature to assess the stability relationship of different polymorphs (Vippagunta et al., 2001).

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Indometacin heat of solution ($\Delta H$) (J/gm)</th>
<th>Nifedipine heat of solution ($\Delta H$) (J/gm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Amorphous (A)</td>
<td>Crystalline (B)</td>
</tr>
<tr>
<td>25°C</td>
<td>-33.20</td>
<td>23.05</td>
</tr>
<tr>
<td>35°C</td>
<td>-36.12</td>
<td>27.49</td>
</tr>
<tr>
<td>40°C</td>
<td>-33.71</td>
<td>32.15</td>
</tr>
</tbody>
</table>

Table 8-1: A list of heat of solution values, for the crystalline and the amorphous states obtained at different isothermal temperatures. Heat of solution was determined using DMF as a solvent for indometacin and nifedipine.

The $\Delta H$ of amorphous sample was exothermic as opposed to endothermic value for crystalline solids (Table 8-1). As the temperature was increased from 25 to 35°C, the $\Delta H$ for the amorphous state became more exothermic whereas for the crystalline state it became more endothermic. Since, $\Delta H_{\text{solvation}}$ should remain same for amorphous and crystalline state at any temperature the value of $\Delta H$ would represent the difference in the energy states of amorphous and crystalline solids or the difference in the lattice
energy $\Delta H_{\text{lattice}}$ between amorphous and crystalline solid. Since internal energy of a solid depends on the molecular mobility, a change in temperature should accompany a change in internal energy mainly in the amorphous state since the molecules in crystalline state are held together by strong intermolecular bonds. It could be noticed from Table 8-1 that the value of $\Delta H$ increased from 56.2 J/gm to 63.6 J/gm for indomethacin and from 58.3 J/gm to 70.6 J/gm for nifedipine with increasing temperature from 25 to 35°C. At 40°C it was anticipated that $\Delta H$ would increase for both indomethacin as well as nifedipine, although for indomethacin increase in temperature from 35°C to 40°C resulted in a slight increase in $\Delta H$ but resulted in decrease in $\Delta H$ for nifedipine. As seen from NIR studies, amorphous nifedipine was highly unstable at higher temperature and a decrease in $\Delta H$ could be correlated to partial crystallisation of amorphous solid.

At 25°C the difference in $\Delta H$ for indomethacin and nifedipine was very similar (56.2 J/gm for indomethacin and 58.3 for nifedipine) whereas at 35°C, $\Delta H$ value was much higher for nifedipine as compared to indomethacin. The higher $\Delta H$ for nifedipine as compared to indomethacin was indicative of higher internal energy and hence higher molecular mobility at both 25°C and 35°C. The effect of increasing temperature was much higher for nifedipine as compared to indomethacin (Figure 8-1). The value of $\Delta H$ would decrease with the lowering of temperature and at certain temperature it would reach zero where the difference between heat of solution of amorphous and crystalline solid would be same; this temperature could correlate to the Kauzmann temperature for amorphous state.

![Figure 8-1](image)

**Figure 8-1:** The difference in heat of solution of amorphous and crystalline state plotted as a function of temperature.
This approach could be further studied using a Micro-SolCal assembly in conjunction with TAM. Micro-SolCal can be used at a range of temperatures and with smaller equilibration time as opposed to fixed few fixed temperatures with longer equilibration time for SolCal. Reducing the equilibration time can significantly reduce the influence of various processes such as relaxation and crystallisation in amorphous solid.

8.2 Study plasticisation effect of organic solvents on amorphous state using IGC

The plasticisation effect of sorbed water on the amorphous material has been studied in detail using IGC (Section 6.0). It was also demonstrated using perfusion unit coupled with TAM that various transitions in amorphous material could be studied as the vapour pressure of different organic solvents was ramped (Section 5.4).

In this work an attempt was made to characterise transitions on the surface of amorphous solid induced by organic vapours such as n-propanol (%RP) using IGC. The injections of decane were made after equilibrating the solids at different values of %RP. This approach suffered a setback as the characterisation of decane peak using flame ionization detector (FID) of IGC was not feasible due to the interference of n-propanol vapours.

The characterisation of transitions on the surface of amorphous solid under the vapour pressure of organic solvents could be very useful in understanding amorphous state of solid.

Further studies are needed in order to study the effect of organic solvents on the amorphous solids using IGC.
8.3 Summary of the thesis

Most of the active substances are hydrophobic hence lack sufficient solubility and bioavailability from the solid dosage form. The amorphous form can exhibit improved solubility over the crystalline form.

Although there are several advantages with the use of the amorphous state, it may be the last choice to be used in solid dosage form. This is mainly due to its tendency to transform into a more stable crystalline state. The studies on amorphous state in are mainly focused around the estimation of,

A. The rate of crystallisation of amorphous state under different conditions.
B. The conditions, which are safe for handling and storage of amorphous state.
C. The approach to stabilize the amorphous state of active e.g. solid dispersions.

The identification of handling and storage conditions (temperature and humidity) is mainly based on glass transition temperature. The recrystallisation tendency of amorphous form is linked to the molecular mobility in the solid state. The prediction of the stability for the amorphous state is based on estimation of molecular mobility and Kauzmann temperature, Tk. It has been generally observed that molecular mobility become insignificant at temperature about 50°C below Tg (Tg-50), so that the recrystallisation can be avoided over the shelf life of the product.

The amorphous state can uptake considerably more amount of water as compared to the crystalline state. The amount of water uptake depends on the RH of the conditions to which the solids are exposed. The absorbed water can act as a plasticizer by lowering the Tg of dry solid. The plasticisation effect of water further complicates the estimation of molecular mobility in the amorphous state making the prediction of Tk unrealistic. Most of the techniques used for characterisation of amorphous solids lack, proper control over RH conditions and this can induce an error in the estimation of Tg.

The techniques used to characterise the amorphous state are no longer limited to differential scanning calorimetry (DSC), X-Ray powder diffraction and dynamic mechanical analysis (DMA). Various techniques such as IR, NIR, NMR, raman, dielectric analysis and thermally stimulated current (TSC) analysis are being employed to further understand the amorphous state.
The aim of the thesis was to identify the techniques to characterise the amorphous state under the controlled conditions of temperature and humidity. These techniques would then be used to identify the conditions for the zero molecular mobility in the amorphous model substances (indomethacin, lactose and nifedipine) selected for the study.

In section 3.0, the characterisation of the amorphous state using DSC was studied. The following results were obtained,

- The estimated value of glass transition for amorphous indomethacin increased with the increase in heating rate. The variation in $T_g$ was used to calculate the fragility index for the amorphous state. Since the absorbed water can affect $T_g$ of the amorphous state and ultimately the estimation of fragility index it is necessary to control the water content of the sample and the RH conditions of measurement.

- The plasticisation effect of absorbed water was studied for amorphous indomethacin and nifedipine using hyper-DSC. In the case of amorphous indomethacin absorbed water lowered the $T_g$ gradually and continually, though the effect was different for amorphous nifedipine. The decrease in $\Delta C_p$ due the sorbed water was more pronounced in the case of amorphous nifedipine as compared to amorphous indomethacin. At higher RH it was difficult to visualize the glass transition from $\Delta C_p$ for amorphous nifedipine. The plasticisation effect of sorbed water on amorphous indomethacin deviated from the Gordon Taylor prediction. The observed deviation could be due to uneven water distribution in the amorphous solid. Hyper-DSC was used successfully to study many attributes of the plasticizing effect of absorbed water for amorphous solids e.g. broadening of $\Delta T_g$, lowering of $\Delta C_p$, multiple glass transitions and collapse of the glassy state.

- StepScan DSC was used to separate the glass transition response from enthalpy recovery. This enabled to study the aging of the amorphous solid at different temperatures below $T_g$. The molecular mobility was estimated using the empirical KWW equation for amorphous state of indomethacin, nifedipine and lactose. A comparison of the molecular mobility among these glasses could be performed in order to study their crystallizing tendency. The lowering of aging temperature below $T_g$ was accompanied with increase in average relaxation time (hence decrease in molecular mobility) for all the glasses. Further lowering of aging temperature resulted in extremely long relaxation times, which could not be measured using the current model. This temperature (with extremely long relaxation times) could be near the zero mobility temperature ($T_k$). The relaxation
process and hence molecular mobility could be successfully studied using StepScan DSC coupled with the empirical KWW equation.

The amorphous state of lactose, indomethacin and nifedipine exhibited different water uptake profiles (section 4.0). Although the BET equation cannot be applied to the water sorption isotherm of amorphous solids, the amorphous lactose exhibited apparent type-II whereas amorphous indomethacin and nifedipine exhibited apparent type-III isotherm. The sorbed water induced transitions in amorphous lactose (e.g. plasticisation, collapse and crystallisation) which could be followed from water sorption isotherm. Although such transitions cannot be denied in amorphous indomethacin they were not as obvious from water sorption isotherm. The sorbed water induced spontaneous crystallisation in amorphous lactose; this was not the case with amorphous indomethacin. Water vapour exposure of amorphous indomethacin at higher temperatures (e.g. 40 and 45°C) was required to induce observable crystallisation. In the case of amorphous indomethacin absorbed water induced a slow crystallisation process which may take several hours to days for the completion. The differences in water vapour interaction of amorphous lactose and indomethacin could be due to the distribution of the sorbed water. Whereas the sorbed water distributes freely throughout the bulk of the solid in the case of amorphous lactose, it may be localised preferentially at the surface for amorphous indomethacin, this differential water distribution may lead to only a part of the solid undergoing transitions as opposed to the bulk transitions.

At any given RH, the amount of water uptake increased with an increase in isothermal temperature for amorphous lactose and amorphous indomethacin (section 4.0). This effect was attributed to an increase in molecular mobility with the increase in temperature. In the case of amorphous nifedipine increasing the isothermal temperature led to a decrease in water uptake at all RH values.

The power output signal obtained from isothermal perfusion microcalorimetry demonstrated similar transitions in amorphous lactose (section 5.2) as observed from the DVS. The range of RH over which transitions were observed in power output signal (obtained by TAM) matched with the range of RH over which a changes were observed in water uptake behaviour of amorphous lactose (obtained by DVS). The value of RH at which the changes were observed in power output signal could be correlated to critical RH (%cRH) for amorphous lactose.

In the case of amorphous indomethacin only glass transition could be observed by using a serial RH ramp at 25°C, as compared to glass transition and crystallisation observed with amorphous lactose. The transitions in amorphous indomethacin viz. glass transition and crystallisation could be followed by using a serial ramp of
(aliphatic) alcohol vapours such as methanol, ethanol and n-propanol. The value of \( \%cRM \) for glass transition (\( \%cRM_g \)) and crystallisation (\( \%cRM_{cry} \)) in amorphous indomethacin could be obtained at different isothermal temperatures. The values of \( \%cRM \) decreased as the temperature was increased. \( \ln\%cRM_g \) and \( \ln\%cRM_{cry} \) exhibited a linear relationship against \( 1/T \). Kauzmann temperature (Tk) calculated for amorphous indomethacin by extrapolating \( \ln\%cRM_g \) and \( \ln\%cRM_{cry} \) against \( 1/T \) line correlated to 2.63 and \(-12.17^\circ C\) respectively. Tk value calculated using the approach described in this work was in agreement with the Tk values reported for amorphous indomethacin using other approaches.

The glass transition phenomenon could be studied using IGC, for amorphous indomethacin (section 6.2). The estimated value of \( T_g \) by IGC (53.3°C) was different than that measured using DSC (44°C) (section 6.3). The different values of \( T_g \) reported for amorphous indomethacin using DSC indicated a clear impact of RH (under which the measurement were performed). The inherent advantage of IGC is its ability to control the RH conditions for the measurement. As a next step the glass transition at the surface of amorphous indomethacin could be estimated as a function of RH. This estimation was based on increasing tailing of the decane peak beyond the glass transition as a plasticisation effect of sorbed water. Using IGC it was possible to demonstrate the preferential plasticisation of the surface of the particle as compared to the bulk (due to RH exposure). This could be useful in studying the plasticisation of hydrophobic substances where the sorbed water is less freely distributed and remains localized mainly at the surface of the particle.

IGC was also effective in studying the plasticisation effect of the sorbed water on hydrophilic substance. The glass transition of amorphous lactose was estimated as a sudden fall in the retention volume of decane due to the plasticisation effect of sorbed water. Using this technique it was possible to estimate the value of critical RH (\( \%cRH_{g} \)) required to lower the \( T_g \) of the amorphous lactose to the experimental temperature. \( \%cRH_{g} \) values could also be estimated as a function of temperature. The values of \( \%cRH_{g} \) estimated using IGC technique matched with those predicted using the Gordon Taylor equation. IGC was also demonstrated to be useful in estimation of mobility onset in amorphous lactose. The values of Tk estimated using different approaches matched closely with each other for amorphous lactose (section 6.5.1). IGC was also useful in studying the phenomenon of collapse in amorphous lactose observed as a sudden fall in pressure drop across the column.

Using NIR (section 7.0) it was possible to distinguish between the amorphous and the crystalline states of indomethacin and nifedipine. The major differences in NIR spectra of the amorphous and crystalline states were noted in the band positions of the functionalities involved in the hydrogen bonding e.g. C=O for indomethacin and C=O
and N-H for nifedipine. It was also noted that the aging in amorphous indometacin was devoid of any major changes in the NIR spectra, whereas for amorphous nifedipine aging was accompanied with significant changes in the NIR spectra. The changes in the NIR spectra of amorphous nifedipine could be correlated to the process of crystallisation.

The various approaches described in this thesis would help in a comprehensive characterisation of the amorphous state using the controlled conditions of temperature as well as RH.
References
Reference List


Angell, C.A., Sare, J. M., and Sare, E. J. Glass transition temperatures for simple molecular liquids and their binary solutions. 1978. Ref Type: Slide


Aso, Y., Yoshioka, S., Kojima, S., 2001. Explanation of the crystallization rate of amorphous nifedipine and phenobarbital from their molecular mobility as measured by (13)C nuclear magnetic resonance relaxation time and the relaxation time obtained from the heating rate dependence of the glass transition temperature. J. Pharm Sci., 90, 798-806.

Aso, Y., Yoshioka, S., Kojima, S., 2000. Relationship between the crystallization rates of amorphous nifedipine, phenobarbital, and flopropione, and their molecular mobility as measured by their enthalpy relaxation and (1)H NMR relaxation times. J. Pharm. Sci., 89, 408-416.


Plambeck, J. A. The Empirical Gas Laws: Vapor Pressure. PSIgate (Physical Sciences Information Gateway). 1996. Ref Type: Electronic Citation


