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Phase Transformations  
in Solid Pharmaceutical Materials  
Studied by  
AFM, ESCA, DSC and SAXS

BY

DENNY MAHLIN



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**Abstract**

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Mixing excipients is a common way to produce pharmaceutical materials with suitable properties for drug formulation. An understanding of the basic mechanisms involved in the formation and transformation of the structures of solid state mixtures is crucial if one is to be able to produce materials with the desired properties in a reliable way.

In the first part of the thesis, the atomic force microscopy (AFM) technique was used to visualise the re-crystallisation of spray-dried amorphous particles comprised of lactose and PVP. The transformation was quantified on a single particle level and analysed with a common kinetic model, the JMAK-equation. The way in which the PVP was incorporated into the particles and the impact this had on their physical stability on exposure to increasing levels of humidity was investigated. The amount and, to a certain extent, the molecular weight of the PVP affected the moisture induced crystallisation of the particles. The inhibition was further discussed in terms of nucleation and growth.

In the second part of the thesis, the formation of phases in solid dispersions of monoolein (MO) in PEGs was studied by the use of SAXS and DSC. Upon solidification of a melt, the components phase separated, resulting in a PEG-rich phase and an MO phase. MO was intercalated into the amorphous domains of the lamellar structure of PEG. A second MO phase appeared in the mixtures where the average molecular weight of PEG was 1500 and 4000 g/mol. It was hypothesised that this second phase was formed in conjunction with the expulsion of MO as the PEG unfolded.

This thesis describes the application of two relatively unexplored solid state techniques on two different solid mixtures of pharmaceutical interest and, in so doing, contributes to the knowledge of phase formation and transformations in the solid state.

*Keywords:* polymer, lipid, lactose, phase transformation, phase formation, crystallisation, AFM, X-ray diffraction, ESCA, DSC, solid dispersion

*Denny Mahlin, Department of Pharmacy, Box 580, Uppsala University, SE-75123 Uppsala, Sweden*

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## List of Papers

This thesis is based on the following papers, which will be referred to by the Roman numerical assigned below:

- I. Mahlin D., Berggren J., Alderborn G. and Engström S. (2004) Moisture-induced surface crystallisation of spray-dried amorphous lactose particles studied by atomic force microscopy. *Journal of Pharmaceutical Sciences* 93 (1) 29-37  
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- II. Mahlin D., Berggren J., Gelius U., Engström S. and Alderborn G. AFM and ESCA characterisation of particle surface topography, composition and crystallisation of spray-dried amorphous composites of lactose and poly vinyl-pyrrolidon (PVP). In manuscript
- III. Mahlin D., Ridell A., Frenning G. and Engström S. (2004) Solid-state characterisation of PEG 4000/monoolein mixtures *Macromolecules* 37 (7) 2665-2667  
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- IV. Mahlin D., Johan U., Ridell A., Frenning G. and Engström S. Characterization of the solid-state structure of PEG/Monoolein mixtures: Influence of polymer molecular weight and addition of peptide drugs. In manuscript



# Contents

Introduction.....	9
Pharmaceutical materials.....	9
Theoretical aspects.....	11
The states of matter.....	11
Mixing components.....	14
Solutions and dispersions.....	16
Kinetics of transformations.....	18
Properties of some excipients.....	21
Lactose — a carbohydrate.....	21
PEG — a semi-crystalline polymer.....	22
Monoolein — a lipid.....	24
Cyclosporine and desmopressin — two peptides.....	25
Solid state methods.....	26
Preparing pharmaceutical solids.....	26
Spray-drying.....	26
Solidification from melt.....	27
Analysing pharmaceutical solids.....	27
Calorimetry.....	27
Microscopy.....	28
X-ray diffraction.....	29
Spectroscopy.....	31
Current research.....	32
Amorphous solid solutions.....	32
Physical stability.....	32
Absorption of humidity.....	34
Lipids in solid dispersions.....	34
Solid lipid nanoparticles.....	35
Cubic phase in drug delivery.....	36
PEG solid dispersions.....	36
Semi-crystalline polymers.....	36
AFM.....	37
This thesis in perspective of current research.....	38

Aims of the thesis.....	41
Preparation and characterisation of the systems .....	42
Lactose / PVP .....	42
Preparation of particles .....	42
AFM.....	42
AFM image analysis .....	43
ESCA .....	43
MO / PEG.....	43
Preparation of solid mixtures.....	43
DSC .....	44
SAXS .....	44
WAXS .....	45
AFM.....	45
Results and discussion .....	45
Lactose / PVP .....	45
Particle surface composition and topography .....	45
Response to increased relative humidity .....	47
Quantification of crystallisation .....	49
MO / PEG.....	53
Phase identification.....	53
Incorporation of MO.....	54
AFM imaging .....	57
Incorporation of peptides.....	57
Summary and Conclusions .....	58
Moisture induced transformations of amorphous particles .....	58
Phase formation in MO / PEG solid mixtures.....	60
Acknowledgements.....	62
References.....	65

## Abbreviations and symbols

$\alpha, \beta$	isomeric forms of lactose
$\alpha, \beta', \beta$	polymorphs of lipids
$\alpha_L$	fraction of surface crystallised, limited growth
$\alpha_E$	fraction of surface crystallised, free growth
$AD$	average deviation
$AD_{cr}$	average deviation of a crystalline surface
$AD_{am}$	average deviation of an amorphous surface
AFM	atomic force microscopy
DSC	differential scanning calorimetry
ESCA	electron spectroscopy for chemical analysis
$\Delta H_m$	melting enthalpy
IR	infra-red radiation
JMAK	Johnson-Mehl-Avrami-Kolmogorov
$k$	crystallisation rate constant
$L$	the long periodicity of a lamellar structure
$l_a$	the length of the amorphous domain
$l_c$	the length of the amorphous domain
$\log P$	the logarithm of the partition coefficient
$m$	JMAK exponent
MO	glyceryl monooleat, monoolein
NMR	nuclear magnetic resonance
PEG	poly (ethylene glycol)
PEO	poly (ethylene oxide)
PVP	poly (N-vinyl pyrrolidon)
RH	relative humidity
SAXS	small angle X-ray scattering
SEDDS	self-emulsifying drug delivery system
SLN	solid lipid nanoparticle
$T_c$	crystallisation temperature
$T_g$	glass transition temperature
$T_K$	Kauzmann temperature
$T_m$	melting temperature
WAXS	wide angle X-ray scattering
wt %	weight percent
$X_{MO}$	total fraction of MO



# Introduction

## Pharmaceutical materials

A pharmaceutical product has many requirements that need to be satisfied if it is to be fully acceptable for use in the treatment of disease. It is rare for an active compound to possess the material properties suitable for administration to a patient without modification. Therefore, in addition to an active compound all pharmaceuticals contain a number of other components each of which contribute to the desired properties of the final product.

Through history the skill of preparing dosage forms by mixing different ingredients has evolved. This has led to a large variety of formulations such as tablets, capsules, ointments and inhalation sprays and powders which are intended for different routes of administration and used for treatment of a variety of diseases.

During the most recent decades the manufacturing of dosage forms has evolved from experience based handicraft to a scientific based and well controlled industrial process. Some of the old dosage forms have become rare, for example, pills and dosage powders. The requirements in terms of safety and economics imposed by patients and authorities benefit other dosage forms and, nowadays, tablets are generally considered to be the most preferred form for self medication.

The traditional preparation methods still constitute the foundation of the manufacturing process of many pharmaceutical products. However, lately the theoretical understanding of the effect of different technical processes on the final performance of the product has increased tremendously. New methods and theories acquired from related areas such as the polymer, ceramic and food sciences have contributed significantly to this knowledge.

There is a widely recognised principle that the traditional, i.e. tried and tested chemicals, such as naturally occurring carbohydrates and lipids, are the first choice when making a drug formulation. The reason for this is obviously that toxic hazards have to be fully examined and eliminated if a new

chemical entity is introduced into the formulation. This procedure is both expensive and time consuming and is therefore avoided whenever possible.

The challenge for persons working with solid pharmaceutical formulation today is often to make the drug available for uptake into the body from the gastrointestinal tract. This can usually be achieved if the dissolution or stability of the compound is increased. Thus, by embedding the compound in functional materials that can hold and release the compound in an appropriate way, these problems can be overcome.

The materials should be traditionally used excipients to avoid the lengthy and expensive approval procedures required for new chemical entities. The desired properties of the carrier material can be obtained by processing existing excipients through the use of various technical procedures and by mixing different components. In this way it is possible to affect the solid state properties of a material without changing its chemical identity. For instance a molecularly disordered material has radically different properties than the ordered, i.e. crystalline, of the same compound. Thus, the mixing of different materials in the solid state can be a way to obtain suitable microenvironments into which the drug can be incorporated. A considerable amount of research is in progress within the area, but some principles and mechanisms for the formation and transformations of the solid structure remain to be revealed. Knowledge of these is necessary to make rational choices about the manufacturing procedures and materials when creating pharmaceutical products.

This thesis focuses on two different kinds of solid mixtures that are thought to be suitable matrixes for the incorporation of drugs. These are used for studying the formation and transformations of solid state structures. The first system is spray-dried amorphous particles that are used to study the stability of against re-crystallisation by addition of a polymer. The other system is a solid dispersion of a lipid in a semi-crystalline polymer, which phase formation upon mixing is investigated.

First a description will be given of some relevant basic phenomena and definitions fundamental to the understanding of the systems under investigation. Thereafter, a short review of the current research within the area and the aims of present work will be presented before a summary is made of the studies done and the conclusions drawn from the work presented here.

## Theoretical aspects

### The states of matter

The number of states the matter can adopt is usually considered to be three; solid, liquid and gas. In a solid, the molecular motion is very slow and the molecules can be found in more or less fixed positions. The **interactions** between the molecules are strong. In a liquid, the interactions are still of considerable strength, but the molecules are able to diffuse very rapidly which makes them change positions continuously, i.e., they have a high **molecular mobility**. In a gas, molecules travel extensive distances without colliding and hence without interacting with other molecules.

A closer look at any material one meets in daily life reveals that the borders between the different states are not always as distinct as one would imagine from the description above. For instance the well ordered **structure** of molecules that is usually associated with a solid can be disturbed so that a structure similar to that of a liquid occurs. One example of this is glass, which is a solid, but which is **disordered** on a molecular scale. In the liquid state, too, one can often find more or less ordered aggregates, i.e. **liquid crystals**, which sometimes make the system appear solid or semi-solid on a macroscopic scale.

The solid state is often **crystalline**. In the crystalline state there is **long range order** on a molecular scale, which means that the molecules are **packed** according to a specific three dimensional pattern that repeats itself in space. A crystal is a particle that can be seen with the naked eye or in a microscope and which consists of molecules arranged in this pattern. The crystal has an outer shape that to some extent reflects the way in which molecules are arranged. Usually a compound can adopt different packing patterns, depending on the method of preparation, whereby different **crystal forms** (i.e. **polymorphs**) are formed.

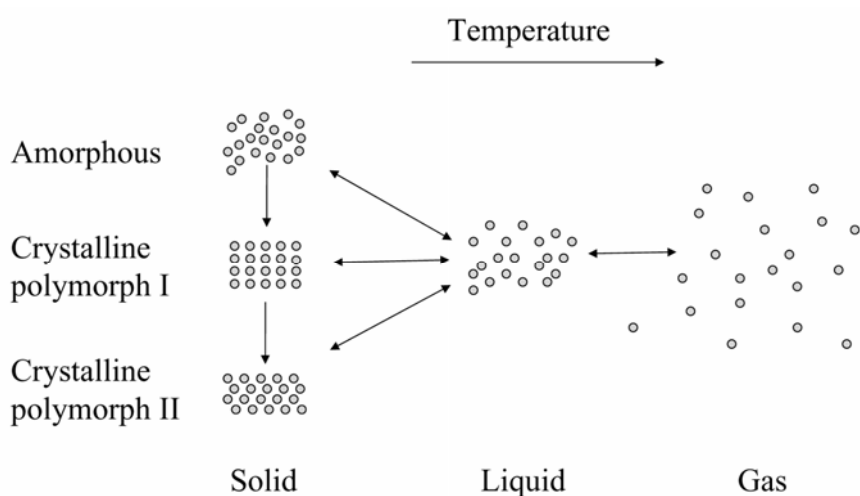
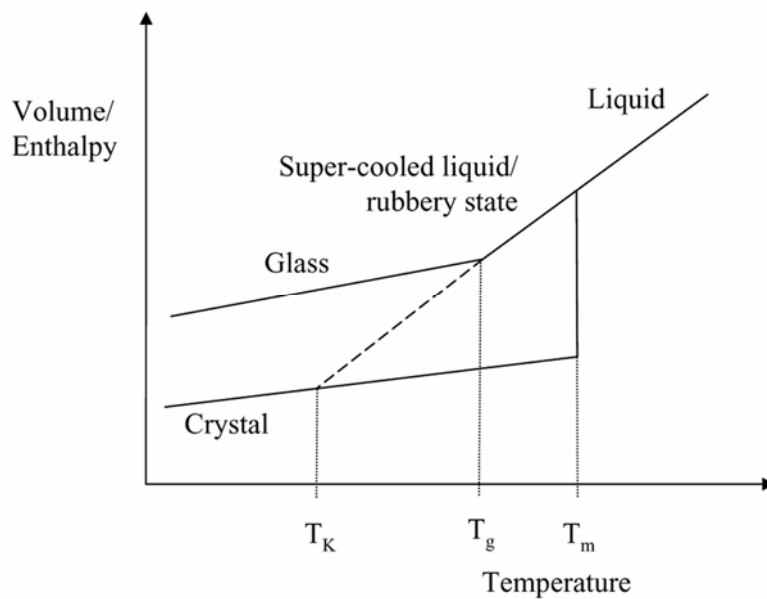


Figure 1. Schematic image of the states of matter on a molecular scale. Depending on the way molecules are arranged within the solid state, amorphous or crystalline forms occur.

Transitions from one state to another can be imposed by changes in the temperature or pressure. The temperature where a liquid is transformed into a crystalline solid is denoted **crystallisation temperature** ( $T_c$ ). Ideally this is the same temperature as where the crystalline solid melts i.e. the **melting temperature** ( $T_m$ ). However, if a liquid is cooled quickly to below this temperature, there is a chance that the molecules will not have sufficient time to arrange themselves in a crystalline state. This is because the molecular mobility gets lower as the temperature decreases, as a result of which the molecules will end up in fixed positions at sufficient low temperatures but with a retained **disordered** structure. What is obtained is usually denoted an **amorphous state**. In contrast to the crystalline state, a particle of an amorphous material can essentially adopt any shape, whence its name, since amorphous means ‘without shape’ which is a characteristic that could not be ascribed an ideal crystal.

An amorphous material has many fundamental properties that are interesting from a scientific viewpoint. Since the molecules are not in positions for optimal interaction with their neighbours, the amorphous state is a highly energetic state, i.e. it is not in thermodynamic **equilibrium**. Consequently, there is a driving force for the molecules to find more favourable interactions and hence to arrange themselves into a crystalline solid. This process is denoted a **re-crystallisation**; the rate at which re-crystallisation takes place is determined by the molecular mobility of the material and, hence, is very slow at temperatures well below the melting temperature.

The volume (and enthalpy) of a liquid/solid system as a function of temperature is shown in Figure 2. When most liquids are cooled, their volume decreases continually (water being an important exception). As it passes below the melting temperature it is thermodynamically favourable for the liquid to be transformed into a crystalline state. Upon a crystallisation the volume of essentially all systems decreases and energy is released from the system in the form of heat. The energy amount released is equal to the **melting enthalpy** ( $\Delta H_m$ ). The crystalline state formed exhibits essentially no change in volume upon a further cooling. However, if no crystallisation occurs then a super-cooled liquid forms which becomes more and more viscous as the temperature and the volume continue to decrease. In theory, at a sufficiently low temperature, the super-cooled liquid would eventually have the same volume as its crystalline counterpart. The temperature at which this hypothetical state would occur is called the Kauzmann temperature ( $T_K$ ) after the person who first made this claim.



*Figure 2.* Schematic illustration of how the volume or enthalpy of a system changes as a function of temperature. Upper solid line shows the pathway for a complete amorphous material while the lower shows the behaviour of a crystalline. Figure adapted from ref. 4.

In a real system this state will never be reached since, at a certain point, the molecules come so close to one another that further volume contraction is not possible. Thus, since the molecules are disordered, the volume attained

at this point will be larger than the volume of the crystalline state. The point at which this happens is called the **glass transition temperature** ( $T_g$ ) and is an important characteristic of an amorphous solid. At this temperature the super-cooled liquid transforms into a **glass** which has many properties that are different from its former state. A glass is a hard, brittle material with a molecular mobility, viscosity, thermal expansivity and heat capacity much lower than the material had above  $T_g$ . This description of the glass transition may be somewhat simplistic and some alternative models exist. Good reviews of these were made recently.<sup>1-4</sup>

## Mixing components

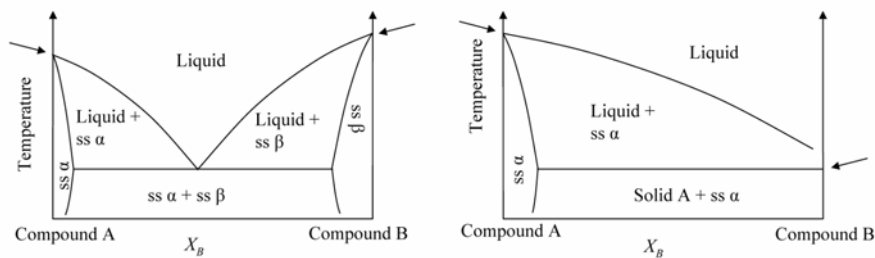
Most materials consist of a **mixture** of two or more **components**. It is hence relevant to here consider the process of mixing. When two components are combined several things may happen. If no chemical reactions occur, the components may either mix spontaneously, whereby molecules from one component are incorporated into the structure of the other, or they might not mix at all with the result that each component forms separate **phases** excluding molecules of the other type. One example of the former is the mixing of water and ethanol which will result in a homogenous liquid, i.e. one single phase. Mixing oil and water, on the other hand, will eventually result in the spontaneous separation of the components, with the water and oil being isolated in two phases. Most common, however, is an intermediate state where a limited amount of one component can be **dissolved**, i.e. molecularly **intercalated**, into the other component. **Phase separation** is the separation of a one phase mixture into two or more phases.

One fundamental driving force for the mixing of two components is the **entropy**. For instance, in a gas the molecules are most likely to be found at random locations at any instance, even if the gas consists of different components. The mixed state is more disordered and more likely to exist. The fact that molecules within the system obtains its most likely configuration can be seen as that it has a driving force for creating a disordered state. This driving force is known as the entropy of mixing and it is generally positive when going from an unmixed to a mixed state, i.e. the disorder increases, upon mixing.

We know from daily life that many liquids and solids remain unmixed. The explanation for why they do not mix is the presence of **interactions** between molecules. If strong interactions, within one of the components, are to be broken upon mixing energy is required, which disfavours mixing. Favourable interactions occurring between the different components in a mixture,

on the other hand, favour mixing of those. If two components that are combined can mix spontaneously or if they will stay separated, is determined by the balance between the changes in interactions between components and the entropy of mixing (i.e. change in disorder). In the ‘theory of regular solutions’ a simple model is used for the calculation of mixing behaviour.

The term phase is defined as a part of a system which is physically and chemically homogenous. From a **phase diagram** one can read out how many phases exist and their variation in **composition** with temperature and overall composition of the system. The appearance of a phase diagram depends on how the components mix, which is a consequence of how they interact on a molecular scale. Two examples of typical phase diagrams for common solid systems can be found in Figure 3. The first type of system shown in the left diagram is called a **eutectic** mixture. In this system the components mix in the liquid state, but not in the solid state with the consequence that melting depression occurs, i.e., the mixture of the components has a lower melting temperature than that of the components unmixed. The other phase diagram in Figure 3 shows the melting behaviour of a **monotectic** system for which the transition from a solid to a liquid upon heating passes through a two phase area. The melting temperature of component B is constant over all compositions (solidus line) while the other decreases monotonously from the melting point of the pure component (liquidus line). This type of melting behaviour is regarded as a limiting case of a eutectic-type where the minimum melting temperature coincide with the melting point of one of the pure components.<sup>5</sup>



*Figure 3.* Two typical phase diagrams for solid two component mixtures. The monotectic (right) is considered to be the limiting case of the eutectic (left). Small amounts of component B may be dissolved in the A whereby solid solution (ss)  $\alpha$  is formed and vice versa for ss  $\beta$ .  $X_B$  denotes mole or weight fraction of component B in the system. Arrows indicate melting temperature of the pure components.

The Flory-Huggins theory for the mixing of polymers is based on the theory of regular solutions and has been successfully applied to many systems for the prediction the phase diagrams of polymer mixtures. It is for instance able

to describe the commonly observed tendency for high molecular weight polymers to phase separate to a larger extent than the low molecular weight counterparts. The Flory-Huggins theory has been the basis for a successful prediction of another important mixing phenomena, the absorption of water by amorphous solids.<sup>6,7</sup>

For the  $T_g$  of a mixed system to be described, another relation that has been derived from the regular solution theory: The Gordon-Taylor equation

$$T_g = \frac{(w_1 \cdot T_{g1}) + (K \cdot w_2 \cdot T_{g2})}{w_1 + (K \cdot w_2)} \quad (1)$$

where  $w$  is the weight of the component and

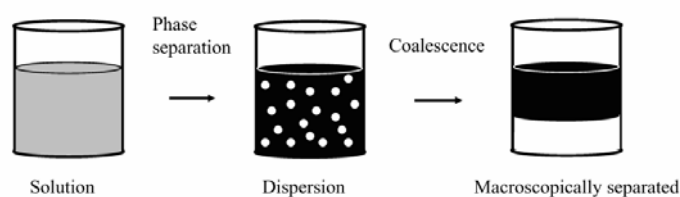
$$K = (\rho_1 \Delta V_2) / (\rho_2 \Delta V_1) \quad (2)$$

$\Delta V$  is the volume expansion at  $T_g$  and  $\rho$  the density of the components. This relation enables  $T_g$  to be calculated for a two component system for all compositions. It is based on the assumption of perfect volume additivity. According to the Gordon-Taylor equation, upon absorption of moisture by an amorphous solid with a high  $T_g$ , a decrease in the  $T_g$  of the system would be expected, as water has a low  $T_g$  (-138°C). And this is indeed true for most pharmaceutical materials, where it is usually said that water **plasticizes** the amorphous solid. This means that the  $T_g$  of the solid is decreased upon water absorption which is a reflection of an increased molecular mobility. This can be understood in terms of a decrease in molecular interactions within the solid imposed by the presence of water.<sup>8</sup>

## Solutions and dispersions

As described above, molecules from one component may be incorporated into the other upon mixing. If this occurs in such a way that the molecules of the incorporated component, the solute, only interact with molecules of the other, the solvent, a **solution** is obtained. If, on the other hand, separate phases are formed, then these phases may either be macroscopically separated or one phase may be **dispersed** in the other. A dispersed system consists of domains, i.e. droplets or particles, of one phase in the other. Milk is a classical example of an **emulsion** (with fat droplets being dispersed in a solution) and paint is an example of a **suspension** (with dye particles being dispersed in a viscous liquid). In pharmaceuticals, dispersed systems are found in, for instance, ointments and pastes.

An important characteristic of a dispersed system is that it contains a large amount of particles or droplets, each one having an interface with its surroundings. If the **interfacial tension** between the phases is large there will be a driving force reducing the total area of these interfaces and spontaneous coalescence of droplets occur. By adding **surface active** compounds, which become enriched in the interfacial regions, the interfacial tension between the phases can be reduced and more stable systems obtained.



*Figure 4.* The general pathway for a transition from solution to macroscopically separated system. The middle illustration shows one phase dispersed in the other. A solid system is likely to be ‘trapped’ in the dispersed state.

**Solid solutions** can be of different types. In a crystalline solid the molecules can be substituted by molecules from another component, resulting in a **substitutional** solid solution. If the molecules of the **solute** are much smaller than the molecules of the **solvent** they may be incorporated in between the packed molecules. Then it is said that an **interstitial** solid solution has been formed. A liquid solution that is solidified without crystallisation taking place forms an **amorphous solid solution**. Owing to restrictions on the relative size and the interactions between the components, the two crystalline types of solid solutions are rare for organic compounds. The amorphous solid solution will be the one formed for the experiments in the first part of this thesis.

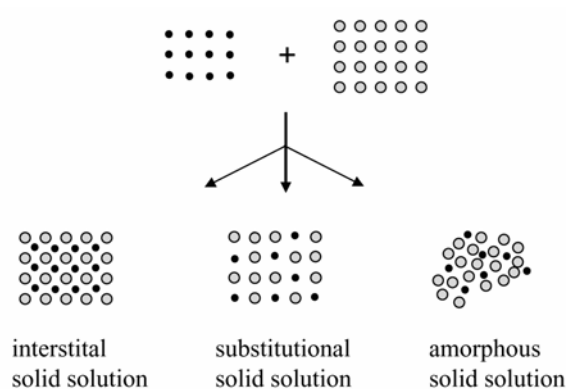


Figure 5. Schematic illustration of typical solid solutions on a molecular scale.

Producing a solid solution of a drug with low water solubility in a solid hydrophilic matrix can increase the release rate of the drug upon dissolution tremendously. However, many pharmaceutical solid mixtures tend to form **solid dispersions** where the components have phase separated into domains. The small particle size obtained (usually in the nano to micrometer range) in this way is usually enough to improve the dissolution rate of a drug.

## Kinetics of transformations

Owing to the very low molecular mobility in solid systems, transitions to thermodynamic equilibrium may take a substantially long time. The rate of transformation to equilibrium is often described by using the term **activation energy**. When going from one state to another the molecules of a system usually have to pass an energetically unfavourable **transition state**. The activation energy is the excess energy, over the average in the system that a molecule has to adopt to be able to reach and pass this state. In a solid few molecules possess the energy required and consequently transitions in the solid state are slow.

Phase separations occur by different mechanisms, which have implications on the rate of transformation. A mixed liquid polymer system, for instance, may phase separate by a mechanism denoted **spinodal decomposition**. In the initial single phase, local concentration fluctuations occur and rapidly increase until a finely dispersed two phase liquid-liquid system has been formed.

Re-crystallisation of the amorphous state often occurs through another mechanism, i.e. by **nucleation and growth**. Nucleation is the process of forming a nucleus, i.e., the initial particle onto which other molecules may adsorb and thereby making the crystal grow. If impurities or small heterogeneous regions are present in the amorphous phase, they may serve as starting points for crystallisation. Such nucleation is termed **heterogeneous nucleation**, whereas nucleation that is equally likely to happen at any location within the system is denoted **homogenous nucleation**.

The **growth rate** of crystals is dependent on the extent to which molecules can reach the surface of the crystal and orientate themselves once there, i.e., on the molecular mobility of the system. Hence the crystal growth rate increases with temperature. The overall crystallisation rate is a combination of the nucleation and crystal growth rate. In Figure 6 a schematic plot of the crystallisation rate as a function of temperature is shown in which the contribution from nucleation and molecular mobility are indicated. The molecular mobility is very low below  $T_g$  but increases rapidly above. The probability of finding molecules arranged into a cluster, i.e. a nucleus, decreases with increasing temperature.<sup>9</sup> Somewhere midway between the  $T_g$  and  $T_m$  the combined effect of the two is at its highest, i.e. there is a maximum in the crystallisation rate.

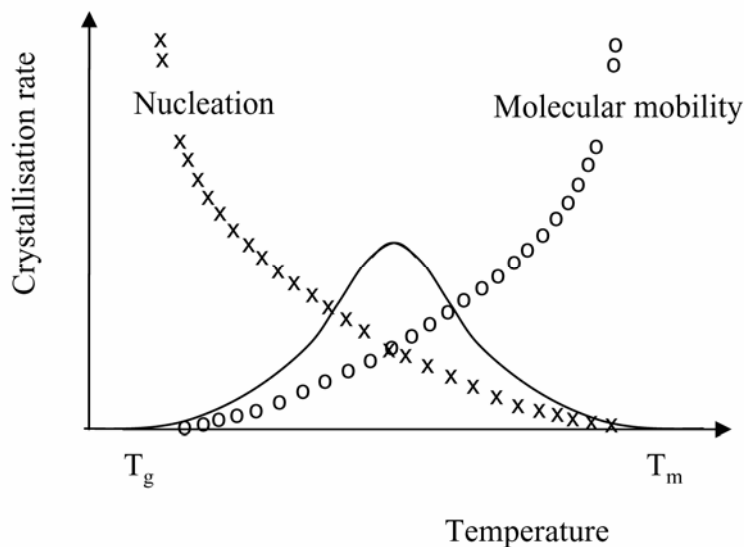


Figure 6. The overall crystallisation rate of an amorphous solid (solid line) as a function of temperature. Figure adapted from ref. 9.

Several equations exist, theoretical as well as empirical, to describe the overall crystallisation of an amorphous solid. The validity of these for a pharmaceutical system has recently been investigated.<sup>10</sup> The most frequently used equations within pharmaceutical research is the Johnson-Mehl-Avrami-Kolmogorov (JMAK) equation.

Avrami proposed a model for phase transformation in 1940 that assumed random formation of the nuclei of a new phase (e.g. the crystalline phase) followed by constant growth in all directions from these nuclei. A way to account for the impingement of the growing domains from different nuclei was also proposed, resulting in the general equation:

$$\alpha_L(t) = 1 - e^{-\alpha_E(t)} \quad (3)$$

where  $\alpha_E(t)$  is a function that describes the imaginary fraction of the transformed phase over time if the crystals are allowed to grow freely, without impinging on other crystals. In a real system, growth is limited and  $\alpha_L(t)$  will eventually reach the value of one as the whole volume becomes crystalline.  $\alpha_L(t)$  can thus be calculated if an expression for  $\alpha_E(t)$  can be found.  $\alpha_E(t)$  can be described in a general way by the integral:

$$\alpha_E(t) = \int_0^t g \left[ \int_{\tau}^t Y(\Theta) d\Theta \right]^m I(\tau) d\tau \quad (4)$$

where  $Y(\Theta)$  is the growth rate for one crystal in each dimension  $m$ ,  $I(\tau)$  the nucleation rate and  $g$  a form factor.  $Y(\Theta)$  is usually constant in each dimension, but the function  $I(\tau)$  depends on the mechanism behind the nucleation. The general solution of the integral combined with Equation 3 gives

$$\alpha_L(t) = 1 - e^{-(kt)^m} \quad (5)$$

where  $k$  is an overall rate constant, and  $m$  will typically have a value between 1 and 4 depending on the number of dimensions in which growth occur and the nucleation rate. This relation can be used to obtain a rate constant for the crystallisation.

## Properties of some excipients

Several different excipients, i.e., compounds which are used to embed the active drug within a dosage form exist and they possess diverse chemical and physical properties.<sup>11</sup> A short description follows of the excipients that will be discussed in this thesis.

### Lactose — a carbohydrate

Lactose is a disaccharide that is one of the most commonly used fillers in tablets and capsules. Because of its many hydroxyl groups (see the chemical formulas in figure 7) it can interact with water through hydrogen bonding and hence dissolves easily in water. Lactose exists in two isomeric forms,  $\alpha$ - and  $\beta$ - lactose and may crystallise into three different crystal forms, the  $\alpha$ -lactose anhydrous or monohydrate and anhydrous  $\beta$ -lactose. Further subclasses have been identified, such as two different anhydrous  $\alpha$ -lactose forms<sup>12</sup> and a crystalline solid solution of  $\alpha$ - and  $\beta$ - lactose<sup>13</sup>. The  $\alpha$  form is predominantly used in solid pharmaceuticals but in water solution mutarotation takes place whereby the  $\beta/\alpha$  ratio increases (to 1.5 at 25°C)<sup>14</sup>. The melting point is for  $\alpha$ -lactose monohydrate 201-202 °C, for the anhydrous  $\alpha$ -lactose 223 °C and for  $\beta$ -lactose 252 °C.<sup>11</sup>

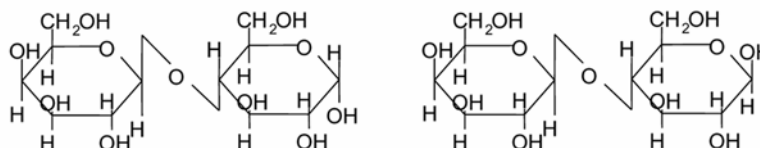


Figure 7. Structural formula of  $\alpha$ -lactose (left) and  $\beta$ -lactose (right)

Lactose is easily made amorphous and is therefore of interest for use as a model compound for studies on re-crystallisation. The amorphous form is hygroscopic and the absorption of moisture lowers the  $T_g$  from 120°C in the dry state to around 13 °C when water is present in 8.5 weight percent (wt %) of the solid. This amount of water can be incorporated by equilibrate amorphous lactose with air at 44.4% relative humidity (RH).<sup>13</sup> This means that, at room temperature, and RHs over  $\sim$  40%, amorphous lactose exists above its  $T_g$  and is in the super-cooled liquid state. In this state re-crystallisation may occur rapidly because of the high molecular mobility of the system.

## PVP — an amorphous polymer

Poly (N-vinyl pyrrolidone) (PVP) is a water soluble polymer that can be obtained in different molecular weights. The smallest repetitive unit, i.e. the monomer, is shown in figure 8. The number of monomer units per molecule in PVP K17, a lower molecular weight fraction (~10,000 g/mol), is approximately 90 whereas PVP K90, a high molecular weight fraction (~1,100,000 g/mol) consists of approximately 10,000 monomer units. As can be seen in the chemical formula, PVP is able to accept hydrogen bonds to one carbonyl oxygen but not to donate any. At high enough concentrations in water solutions PVP adsorbs to the liquid-air interface.<sup>15</sup>

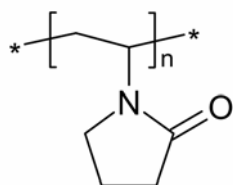
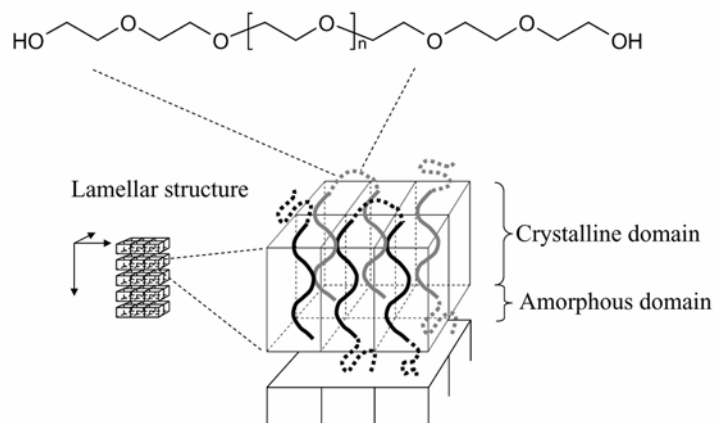


Figure 8. Structural formula for the PVP monomer unit.

In the solid state, PVP is amorphous and has no definite fusion temperature. It is, however, chemically degraded at higher temperatures (~250°C). PVP is mainly used in solid formulations for coating and as a binder in wet granulation. It increases the viscosity of water solutions and is hence used for adjusting consistency and stabilise emulsions.

## PEG — a semi-crystalline polymer

Like PVP, poly (ethylene glycol) (PEG) is water soluble and is available in a wide range of average molecular weights. The PEG polymer chain consists of the same monomer unit as poly (ethyleneoxide) (PEO). The monomer unit is shown within brackets in figure 9. The chemical structure of the polymer chain imposes interesting solubility behaviour in water, i.e., the solubility decreases as the temperature is increased. The average molecular weights mostly used in pharmaceuticals vary from 200 to 20 000 g/mol where the low molecular weights (up to 600 g/mol) are liquids at room temperature.<sup>11</sup>



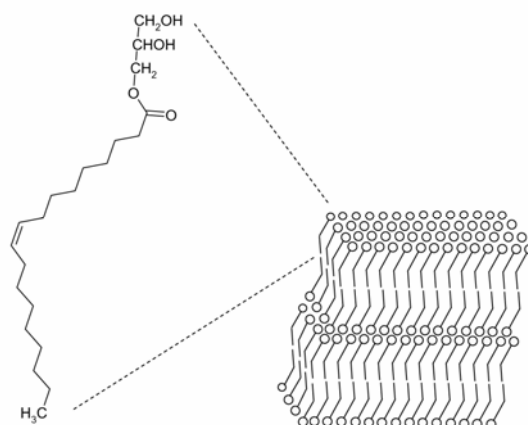
*Figure 9.* Schematic illustration of the PEG lamellar structure. The extension in z direction is negligible compared to in the x and y directions.

In the solid state PEG is semi-crystalline. Its molecules crystallise in a helical structure packed in layers, so called lamellae, with the axis of the helix perpendicular to the packing planes. The helix of the PEGs can be folded once or more times upon crystallisation. The folds and the ends of the molecules constitute the volume in between crystalline the lamellae, whereby an alternating amorphous and crystalline structure is obtained i.e. a lamellar structure (see Figure 9). Upon crystallisation from a melt, longer polymer chains are more likely to fold. After primary crystallisation, an unfolding occurs which is a less demanding process for short polymer chains. The final degree of folding is hence determined by the molecular weight. PEGs larger than 4000 g/mol are found to be stable in forms that have been folded once or even more as the molecular weight increases.<sup>16</sup> The lamellae form crystallites that are arranged into macroscopically visible spherulites.

Solid PEGs have relatively low melting points, e.g. 50-58°C for PEG 4000 and 60-62°C for PEG 8000 which make them useful in the preparation of solid dispersions.<sup>11</sup> However, their poor compaction properties make them unsuitable as a major component in tablets but are useful as binders in combination with other excipients. In aqueous solutions PEG, as most water soluble polymers, increases viscosity and have, in addition, the ability to enhance aqueous solubility of drugs.

## Monoolein — a lipid

1-glyceryl monooleate (monoolein, MO) is a non-soluble polar lipid that appears in the digestion of ordinary triglycerides from nutritional fats and oils. The chemical structure can be found in Figure 10. MO swells and forms liquid crystals upon contact with water. The cubic phase and its dispersed form (cubosomes) have gained scientific interest.<sup>17</sup>



*Figure 10.* The chemical formula and a model of the lamellar packing of monoolein in the solid state.

In the solid state, monoolein adopts a lamellar structure, giving the hydrophilic ‘head-group’ (the glyceryl group) and the hydrophobic ‘tail’ (the hydrocarbon chain) of the molecules the opportunity to interact favourably (see Figure 10). A melted lipid that solidifies usually passes through different crystal forms before it reaches the most stable form. The main forms are denoted  $\alpha$ ,  $\beta'$  and  $\beta$ . The molecules in the  $\alpha$  form are in fixed positions, but rotates around the axis of the hydrocarbon chain. Within the planes the molecules are packed in a hexagonal structure. In the  $\beta'$  and  $\beta$  form the rotation has ceased and the difference between those polymorphs is only the packing within the plane (orthorhombic and triclinic, respectively).<sup>18</sup> The polymorphs differ in melting point (25, 31 and 34 °C, respectively)<sup>19</sup> and WAXS diffraction pattern. However the lamellar thickness is unaffected by the transitions between the different forms and is therefore not detectable with SAXS.<sup>18</sup>

## Cyclosporine and desmopressin — two peptides

Peptides are small proteins no larger than 100 amino acids. The abundant peptide bond in the chemical structure makes all peptides susceptible to chemical degradation in the gut and intestine. Because of this their bioavailability is very poor after oral administration.

Cyclosporine is an immunosuppressive agent used for the treatment of autoimmune diseases. Sandimmune® Neoral® is a commercial product of cyclosporine, which has a self emulsifying carrier to improve its bioavailability.<sup>20</sup> Many of the amino acids in the chemical structure have hydrophobic side-chains, which make this compound hydrophobic and hence poorly soluble in water ( $\log P > 1$ ).

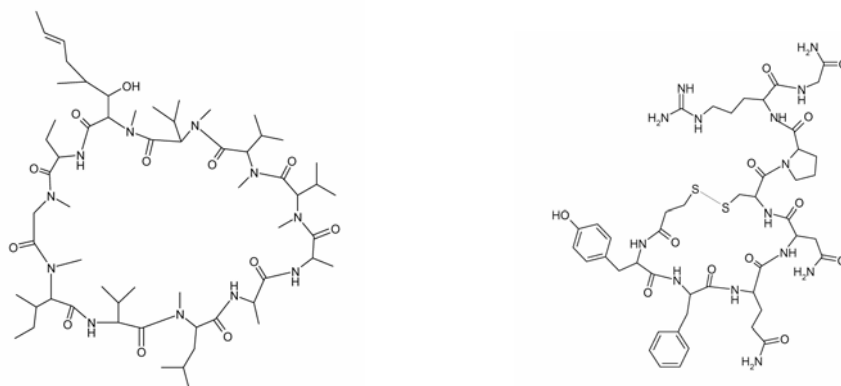


Figure 11. Structural formula of cyclosporine and desmopressin (right).

In contrast, desmopressin, a peptide used for the treatment of incontinence, is a hydrophilic ( $\log P < -4$ )<sup>21</sup>. Desmopressin is found in the commercial product Minirin® which in form of a tablet displays a very poor bioavailability (0.1 – 0.16%). This can be attributed to the chemical instability in the gastro-intestinal fluids and poor transport through the gut wall.<sup>22</sup>

## Solid state methods

### Preparing pharmaceutical solids

Often the same kind of techniques can be used for the preparation of amorphous solids that also are used for producing solid dispersions. At least, they may be categorised in the same way. Typically, there are three different principles: Dissolving solid materials and then removing the solvent by evaporation, a rapid solidification of a melt or the milling of dry powders. The latter can at least partially induce disorder in a material and cause mixing of components on a molecular scale. These are the basic principles underlying a variety of techniques that have been developed for the preparation of pharmaceutical solids. The summary below concerns only the methods relevant for this thesis.

#### Spray-drying

In spray-drying, freeze-drying and other solvent evaporation methods mixing is achieved by dissolving the components in a common solvent, preferable water. The techniques differ in what way the solvent is removed from the material. In spray-drying a solution, an emulsion or a suspension is sprayed into a hot stream of air, usually at 100-200°C. The liquid droplets formed upon spraying are dried rapidly within seconds of the atomization. The time for crust formation, i.e. the transformation from a liquid solution to a solid in the outer regions of the particle, is much shorter than one second.<sup>23</sup> The rapid evaporation makes this method useful for creating amorphous solid solutions with well defined particle properties. It is for instance possible to control the size of the particles formed by variation in the droplet size and the concentration of the solute in the spray-drying liquid.<sup>24</sup>

## Solidification from melt

The co-melting of components at elevated temperatures followed by solidification through cooling, which should be rapid (quench cooling) if an amorphous state is to be obtained, is a commonly used method, at least on a laboratory scale. The major drawback is the risk of degradation of heat-sensitive compounds. Despite this drawback, this method of preparation has regained new interest since a hot-melt extrusion process has been shown to be useful for the manufacture of pharmaceutical solids.<sup>25,26</sup> In the hot-melt extrusion method, used within the polymer science for years, the components are heated, mixed and extruded, essentially simultaneously, minimizing the time that the materials are exposed to heat.

## Analysing pharmaceutical solids

A variety of techniques are used to study solid pharmaceuticals. Powder functional properties, stability and biopharmaceutical performance are important issues to evaluate during development of a drug formulation. For instance by studying the release pattern of a drug from a formulation in water or simulated intestinal fluids, indications of how the system works *in vivo* can be obtained as well as indirect information on the solid state structure. However, since this thesis is aiming to study basic mechanisms for solid state structure formation more direct solid state methods are appropriate. The summation done below will only concern the methods relevant for this thesis.

### Calorimetry

Calorimetric methods are based on measuring the heat released or taken up by a system in association with different chemical reactions or phase transitions, such as melting and crystallisation.

### DSC

In differential scanning calorimetry (DSC) the enthalpy change, i.e., the heat transfer, to or from a system is measured as the temperature of the sample is increased (or decreased) constantly. The energy that is required to increase the temperature of the sample can be determined, which enables a calculation to be made of the heat capacity of a sample. Hence, the change in heat capacity that an amorphous sample displays as it passes through the glass

transition can be detected and, in that way,  $T_g$  determined. Since a phase transition involves the breaking and formation of bonds, energy will be released or taken up as such event occurs, enabling it to be detected. In this way, the enthalpy of transitions, e.g.  $\Delta H_m$ , can be quantified.

### **Microcalorimetry**

Microcalorimetry is an iso-thermal method, which means that during the measurement the temperature is kept constant. Measurable transitions are induced by other means, for instance by increasing the humidity of the sample. The absorption of humidity into the sample is itself an exothermic (heat releasing) process and can hence be studied by microcalorimetry. Smaller heat transfers can be detected than are measurable with DSC, whence its name ('micro-').

### **Microscopy**

The classical microscope, the light microscope, is one of the earliest research instruments used. Over time techniques making it possible to visualise very small objects have evolved. One of the latest inventions for this purpose is the scanning probe technique which is just a couple of decades old.

### **AFM**

Atomic force microscopy (AFM) also known as scanning force microscopy (SFM) is a scanning probe technique which visualises the surface structure by scanning a tip over a sample while a laser measures the deflection of the cantilever onto which the tip is attached. In this way the surface topography can be detected on a nano-scale and a 3D image of the sample obtained. Moreover, AFM may be run in different modes to obtain indications of some surface-related properties such as friction and rheology. In the AC ('tapping') mode, the tip is oscillated over the surface during scanning. The amplitude of the oscillation is detected and, when interactions occur, the amplitude and the oscillation phase will be affected. In this way, phase imaging can provide an image on the nano-scale of the different domains on a solid surface, provided that they interact with the tip in different ways.

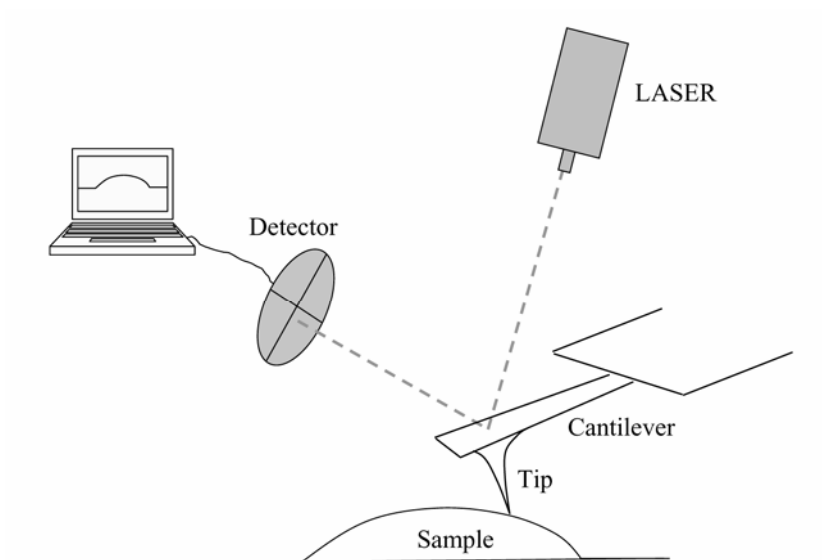


Figure 12. A schematic illustration of the essential parts of an AFM.

AFM has found applications in many areas, such as biological, polymer and materials sciences. The technique also has considerable potential in the field of pharmaceuticals. It offers a unique means of scanning unprepared samples in different environments, including liquids, and at controlled temperature and relative humidity. A possible application could thus be the examination of solid systems during phase transformations, e.g., during crystallisation of amorphous materials.

## X-ray diffraction

The scattering of light can be used to study a wide range of both liquid and solid systems. Detectable diffraction phenomena occur when a crystalline material which contains nano-scale periodicities within its structure, scatter electromagnetic radiation with wavelengths in the X-ray region.

## WAXS

When a crystalline sample is irradiated with X-rays it is producing a diffraction pattern that can be detected and analysed. Depending on whether the sample is a single crystal or a powder (consisting of many small crystals, randomly oriented) the diffraction pattern has to be detected and analysed in different ways. Preparation of single crystals of organic compounds is often problematic, which has made powder diffraction most used for pharmaceuti-

cals. In powder diffraction, a 'diffractogram' is obtained showing with what intensities and what angles from the incoming beam the light is diffracted. From this conclusions can be made about the crystal form of the solid. Short periodicities in a crystal give rise to diffracted light at relatively large angles. In wide angle X-ray scattering/diffraction (WAXS/WAXD) these small distances are identified. Further information of non-perfect packed solids can be obtained. Small crystallites or the occurrence of non-perfect periodicities broadens the diffracted peaks and if there are amorphous domains within the sample broad bumps appear in the diffractogram at the expense of the sharp peaks characteristic of a crystalline material. Powder diffraction is one of the most important solid state characterisation methods within the pharmaceutical area.

### SAXS

With the WAXS technique, structure related spacings that are found within most kinds of crystals can be studied. These spacings correspond to distances between atomic planes within the crystalline structure; typically 1 – 50 Å. Longer spacing give rise to diffracted light at small angles. The detection then has to be made at greater distances from the sample to increase the resolution so that the diffracted light can be separated from the primary beam. This is the set up used for the small angle X-ray scattering (SAXS) technique, which can be used to study repetitive spacings from 10 to several hundred Ångströms. This often corresponds to the dimensions of a lamellae of semi-crystalline polymers and liquid crystals and, consequently, the technique is used for studying such systems. In SAXS the 'scattering vector' is often used to described the scattering instead of the 'scattering angle'.

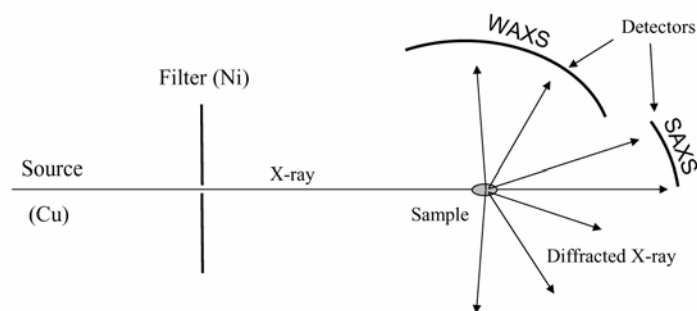


Figure 13. A schematic illustration of the essential components in X-ray diffraction.

A common way to analyse diffraction data from semi-crystalline polymers is to calculate the one dimension correlation function. This is the cosine trans-

form (a half sided Fourier transform) of the Lorentz corrected intensities, i.e., the background corrected intensity multiplied by the scattering vector squared. A more thorough description of the procedure is found in Paper III of the thesis. The information obtained is the length of the overall periodicity ( $L$ ) of the lamellar structure and the length of the crystalline ( $l_c$ ) and amorphous domains ( $l_a$ ) in the direction of the lamellar packing in a semi-crystalline polymer (see Figure 9).

## Spectroscopy

If a material is exposed to radiation it may absorb the energy from the radiation in several different ways. For instance,  $\pi$ -electrons in an aromatic structure can be excited to a higher energy level by ultra-violet light. The vibrational energy of a chemical bond can be increased by absorption of infra-red (IR) light. The energy of the light absorbed or of the light transmitted from the material as it relaxes back to its initial state can be detected and can provide useful information about the material. In nuclear magnetic resonance spectroscopy (NMR) as well as IR spectroscopy absorbed energies are determined (upon exposure to radiofrequency radiation and IR light, respectively). Measurement of these energies enables, for instance, determination of the components present, their amount and interactions taking place, within the material.

## ESCA

The electron spectroscopy for chemical analysis (ESCA) technique is founded on an analysis of the kinetic energy of the photoelectrons emitted from a sample exposed to monochromatic X-ray radiation. Each element present in the sample releases electrons with specific kinetic energies. As electrons only can travel a very short distance in a solid sample without losing energy, only electrons originating from a thin surface layer — down to about 10 nm — give rise to the characteristic spectral lines. Photoelectrons from deeper layers lose their kinetic energy and form a continuous background on the low kinetic energy side of each characteristic line. However, the main part of the ESCA line intensity originates from just the top 3 to 4 nm. Furthermore, when flat and very smooth samples are studied at a grazing emission angle, the main signal originates from an even thinner surface layer, of approximately 1 nm. Consequently, the ESCA method is extremely surface specific. Since the measurements have to be conducted in ultra-high vacuum, samples containing volatile components, such as water, are not suitable. Organic materials usually have to be dried extensively before measurements can take place.

## Current research

The concept of embedding a drug in a carrier by formation of either a solid dispersion or an amorphous solid solution is a well established procedure within the field of solid formulation. However, there are only a few products on the market today which are based on these kinds of systems, largely because of instability of these products on storage, which is a consequence of the systems not being in equilibrium.

Despite this, many research groups, both within academia and in the pharmaceutical industry, have put a great deal of effort and are investing resources on research in this area. The obvious advantages that could be gained if the stability problem were to be solved still make these formulation concepts appealing. Where very few alternatives exist they could provide a means to formulate drugs with very low solubility and poor stability in solid dosage forms intended for oral delivery.

## Amorphous solid solutions

### Physical stability

A promising approach to increase the physical stability of amorphous solid solutions has been the addition of polymers possessing a high  $T_g$  to the amorphous material. It was clear from early studies that the addition of a high  $T_g$  polymer would increase the  $T_g$  of the system as a whole<sup>27</sup>. The molecular mobility of an amorphous material is much lower below  $T_g$  and hence an increase in the  $T_g$  was expected to result in a decrease in the recrystallisation rate. The concept of stabilising the amorphous state through the addition of a polymer was initially presented by Zografi and co-workers in 1996<sup>28</sup> and has since then been applied to several systems. Examples of polymers are Ficoll (a cross-linked synthetic polysaccharide)<sup>28</sup>, dextran<sup>29,30</sup>, PVP<sup>28,31</sup>, hydroxypropylcellulose<sup>32</sup> and poly(vinylpyrrolidone-co-vinylacetate)<sup>33</sup> which have been used to stabilise drugs such as indomethacin<sup>34</sup> and nifedipin<sup>35</sup> and sugars, e.g. lactose<sup>8,31,36,37</sup> and sucrose<sup>38,39</sup>. It has

also been shown that adding PEG to lactose had a negative effect on the stability since PEG has a much lower  $T_g$  than lactose<sup>36,40</sup>. As a rule of thumb for the stabilisation of pharmaceuticals, Hancock et al (1996) proposed that the  $T_g$  of a solid pharmaceutical product should be at least 50 °C above the storage temperature.<sup>41</sup> This corresponds to storage at the Kauzmann temperature.

Already from the early crystallisation studies it could be concluded that the expected relation between inhibited crystallisation and an increase in  $T_g$  was poor for some systems. Particularly when PVP was used as stabiliser, the crystallisation could be significantly retarded while the  $T_g$  of the system remained unaffected.<sup>28,31</sup> A probable explanation for this discrepancy is the occurrence of an interaction between the polymer and the amorphous component upon mixing the components.<sup>33,42</sup> The consequence of this interaction seems to be twofold: a deviation from the ideal Gordon-Taylor equation<sup>30</sup> and excessive stability in comparison with the increase in stability anticipated from the increase in  $T_g$ .<sup>31</sup>

With the intention of gaining a better understanding of these issues and of identifying a more suitable parameter than  $T_g$  to indicate physical stability, a number of alternative methods have been evaluated. Theories emerging from more fundamental research on the nature of the amorphous state have led to the use molecular mobility and of relaxation times as alternative parameters. These can be determined using various methods such as DSC,<sup>41,43</sup> NMR<sup>44</sup> and dielectric measurements<sup>35,45,46</sup> which show that detailed information can be obtained on mobility and interactions in the amorphous state. The importance of nucleation has been evaluated.<sup>47,48</sup> Nucleation was, for instance, found to determine which polymorph of indomethacin was formed. In a recent study molecular mobility and relaxation rates upon addition of PVP have been determined and found useful both for prediction of stability and giving information on interactions taking place in those amorphous mixtures.<sup>35,49</sup>

The importance of particle size on the re-crystallisation rate of amorphous indomethacin has been emphasised by Crowley and Zografi<sup>34</sup> It was proposed that the particle surface is likely to possess a large number of nucleation sites and that crystallisation mostly originated from there. Hence, a system with a large surface area, i.e. consisting of smaller particles, would crystallise more rapidly.

## Absorption of humidity

Another important issue is in what way humidity influences an amorphous material and affects its stability. Microcalorimetry gives information on the absorption and desorption of water in association with crystallisation.<sup>37,50-52</sup> Sorption studies are, however, usually conducted gravimetrically both when the equilibrium moisture contents are studied and when kinetics of adsorption into amorphous solids are of interest.<sup>53-55</sup> This method has revealed, for instance, that physical mixtures of lactose with 'internal desiccants' such as PVP displays increased physical stability as the RH increases.<sup>56</sup> The improved stability against humidity-induced re-crystallisation of amorphous solids brought about by incorporating PVP has been evaluated in several publications.<sup>28,31,32,39</sup> A systematic investigation of the stability of spray-dried composite particles has recently been performed in a PhD thesis, where the influence of RH, PVP molecular weight and amount on storage stability and compaction properties were studied.<sup>57</sup>

The effect of moisture on the molecular interactions in the amorphous state has recently been investigated in a study where IR spectroscopy was used to show that the hydrogen bonding within sucrose and lactose decreased as water was absorbed.<sup>8</sup> In another study it was concluded that sucrose and PVP interact, probably by hydrogen bonding, in a solid solution of sucrose and PVP.<sup>49</sup> No decrease in the interaction strength between the sucrose and the polymer could be detected as the moisture content was increased. However, the RH of these studies was very low (11 and 22%, respectively) and may not reflect the conditions above  $T_g$ .

Some interesting examples on the impact of moisture on amorphous materials have been published by Buckton and co-workers<sup>55,58,59</sup>. Both the rate of water desorption and the re-crystallisation rate of freeze-dried lactose were affected by degree of collapse of the amorphous material. It was concluded that a collapsed material crystallises at lower temperatures than an un-collapsed one.<sup>59</sup>

## Lipids in solid dispersions

The theory on the physical stability of the amorphous phase is equally applicable to solid dispersions since there are usually domains within the structure that are amorphous, and hence susceptible to solid state transformations. The large interface to bulk ratio that a dispersed system possesses further contributes to its instability.

A survey of recent publications reveals that many different aspects of solid dispersions have been subjected to examination. Furthermore, some extensive reviews focusing on aspects of drug formulation have been published within the last few years.<sup>60-62</sup> However, since the latter part of this thesis specifically concerns a lipid dispersed in solid PEG, the following section will be devoted to research relevant to this mixture.

The idea of creating a self-emulsifying drug delivery system (SEDDS) that forms a liquid microemulsion upon dissolution in water has been around for some time and resulted in the commercial formulation of cyclosporine, Sandimmune® Neoral®.<sup>20</sup> As the number of drugs with poor solubility and stability (e.g. proteins and peptides) increase, interest in a similar system denoted 'dry emulsions' has emerged.<sup>63,64</sup> Here spray-drying or freeze-drying is used to obtain a solid enclosing pre-dispersed fatty domains. In principle, this results in the same type of system as spray-dried or freeze-dried cubosomes or solid lipid nanoparticles (SLNs).

## Solid lipid nanoparticles

In lipid formulations, hydrophobic drugs are dissolved in an oil or a solid fat which, upon ingestion, will be digested and hence able to release the drug for absorption from the intestine. To circumvent inconsistency in the bioavailability, owing to variations in digestion before and after food intake and amongst individuals, it has been proposed that SLNs could be used for oral delivery of poorly soluble compounds.<sup>65,66</sup> SLNs are solid lipids dispersed to nano-sized (< 1000 nm) particles to give better controlled drug release properties than ordinary lipid formulations. For parenteral administration SLN dispersions are thought to have superior biocompatible properties to polymeric nanoparticles and better stability than liposomes.<sup>61</sup> Peptides such as cyclosporine have successfully been incorporated in these particles.<sup>67,68</sup> Recent studies have shown that spray-drying and freeze-drying of SLNs is possible, which, in addition to the improved storage properties of parenteral SLN formulations, would enable SLNs to be incorporated in solid oral dosage forms.<sup>60,69</sup>

SLN are usually prepared by dispersing the lipid in the liquid state at elevated temperatures. The fatty emulsion droplets formed are then able to dissolve an added hydrophobic drug. Subsequent crystallisation of the lipid via the  $\alpha$  and  $\beta'$  - to the stable  $\beta$ -form usually results in expulsion of the incorporated drug during storage.<sup>61</sup> Addition of lipids with different chain-lengths can promote the stabilisation of a more disordered state of the solid lipid.<sup>70,71</sup> These kinds of systems are denoted nano-structured lipid carriers (NLC) and seem to be more suitable for drug incorporation than ordinary SLNs.

## Cubic phase in drug delivery

Some polar lipids or mixtures of lipids swell upon contact with water and form a cubic phase i.e. a liquid crystal in which lipid and water are arranged in a cubic structure of nanometre dimensions.<sup>72</sup> Both the aqueous and lipid domains within the structure are continuous which provides unique opportunities to incorporate many different kinds of compounds. Thus, this phase is potentially useful for several applications such as protein crystallisation<sup>73</sup> and drug delivery.<sup>17,74</sup> It has been shown that a variety of peptides can be incorporated into the cubic phase where they become protected from degradation of simulated intestinal fluids.<sup>74</sup> Dispersions of the cubic phase, i.e. cubosomes, could provide similar advantages as the dispersed solid lipids and have, therefore, been proposed as drug delivery vehicles.<sup>75-77</sup>

## PEG solid dispersions

The relatively low melting point makes PEG a suitable matrix for the preparation of solid dispersions by co-melting. The use and properties of PEG in solid dispersions are comprehensively described in the literature.<sup>5,25,78</sup> Some studies have focused on the influence of molecular weight on the release pattern of model compounds.<sup>25,79</sup> The ability to dissolve the drugs to form a solid solution is an important issue here. However, the release process is complex and therefore difficult to predict.<sup>25,79</sup> Improvement in the ability to dissolve certain substances, for instance griseofulvin and indomethacin into solid PEGs, have been the focus of numerous studies.<sup>80-84</sup> One interesting result in this respect was that the addition of a variety of surfactants and cyclodextrines can be useful for these purposes. The solid state mixing behaviour of lipids with PEG have not been as extensively examined but recently the melting behaviour of different fatty acids in mixtures with PEG has been investigated.<sup>85</sup> Another interesting system is the 'NanoCrystals' where drugs are dispersed in liquid PEG which solidifies after filling into capsules.<sup>62</sup> The advantage was claimed to be the preparation of nanoparticles in a non-aqueous medium.

## Semi-crystalline polymers

Semi-crystalline polymers such as PEO and poly(oxymethylene) have recently been studied by SAXS.<sup>86-89</sup> In these studies a second component was added to the semi-crystalline polymer and its influence on lamellar packing observed. In these studies the one dimensional correlation function was calculated and used to study polymer folding within the lamellae. The results

indicate that added components is being incorporated into the amorphous domains of the lamellar or they may be expelled from the lamellar structure.<sup>86</sup>

## AFM

AFM is a recently introduced method within the pharmaceutical field. Its most obvious application is probably in the visualisation of surfaces and of macromolecules attached to them. Developments are taking place rapidly and applications that seize upon the ability to register interactions between the tip of the cantilever and the samples continually appear in the literature. A few examples will be presented here.

Through the AFM detection of different kinds of surface related properties, such as friction, it has proved to be possible to differentiate between crystal forms of a drug in a mixed powder sample.<sup>90</sup> Moisture sorption on lactose<sup>91</sup> has been quantified by this technique (colloidal probe), as have friction and adhesion on a micro scale<sup>92,93</sup> However the literature on the use of AFM for the study of the crystallisation processes is limited. Trojak and co-workers reported that the surface roughness increased significantly as a film of amorphous felodipine crystallised<sup>94</sup> and Beekmans and co-workers used AFM to study the kinetics of crystal melting of poly(ethylene oxide)<sup>95</sup>. The surface of crystals in solution and of crystallising amorphous lactose have been visualised by AFM.<sup>96</sup> Thus, a variety of applications have been explored lately and, in addition, the technique is continually being developed. In this respect, the micro-thermal analysis is one of the developments that seems to have potential in the field of pharmaceutical materials research because of its possibility to induce melting locally and thereby making it possible to identify domains within a solid dispersion, for instance.<sup>97-99</sup>

## This thesis in perspective of current research

During the last decade, there has been a tremendously rapid development of methods for identification of chemical and biological compounds to be used as new drugs. These methods often generate drug candidates that are very potent in terms of biological activity but at the same time problematic from a biopharmaceutical viewpoint. Consequently, the focus of solid formulation research has shifted lately towards finding ways to improve the solubility of hydrophobic substances and the stability of proteins and peptides. The challenge is to create a beneficial environment within the solid structure that can protect and release these substances upon storage and usage.

The literature survey conducted in the previous part of the thesis revealed that several concepts have been put forward for handling these issues. Amongst these, embedding the drug in an amorphous material may be advantageous. Other approaches mentioned have been the preparation of solid dispersions and lipid formulations. These solutions appear to be beneficial in the sense that the materials to be used can easily be chosen from already approved excipients and food constituents. For instance, different kinds of carbohydrates are used for protein formulation and naturally occurring lipids for formulating insoluble compounds.

It is well recognised that the mixing of different excipients by a suitable method can give rise to materials with functional properties for drug formulation. However, it is not always easy to predict the properties of a mixture from the properties of the individual components. Trial and error could be reduced if a deeper understanding of the formation and transformations of the solid state structure were to be obtained. This kind of knowledge is crucial if one is to make a rational choice of components and preparation technique for solid formulations.

In this context, many polymers are of particular interest and display several useful qualities. In addition to the properties that they possess because of the chemical structure of their monomer units, their molecular weight, i.e., the chain length is of importance for their solid state behaviour and for determining the way in which they will mix with other components.

A method that has emerged and appears to be useful for creating functional materials is the spray-drying technique which can generate an amorphous material in the form of particles. However, as was obvious from the literature review, a major concern is the poor physical stability of the amorphous state. The theory of the physical stability of amorphous materials is well established because of the numerous studies that have been conducted in recent years, but the stabilising effect of co-mixed polymers is not fully understood, not at least in particulate materials. In order to gain further knowledge, it would be advantageous to implement methods that can allow studies to be conducted on a single particle level. One method that can meet these demands is atomic force microscopy, which has gained increased interest in this field lately. The technique has a high resolution and can be used to probe a particle surface while re-crystallisation occurs. Of particular interest for AFM studies of such a phenomenon on single particle level is the stabilising effect of PVP on lactose, since it could provide information on the crystallisation mechanism of a bulk system which consists of numerous particles. A considerable amount of studies have been done on bulk samples of these compounds by other methods.

AFM could also be useful for the characterisation of solid dispersions owing to its ability to detect nano- to micro metre sized domains by the use of phase imaging. The possibility of preparing solid dispersions and solutions by co-melting components has gained new interest lately, as a consequence of the transfer of the hot-melt-extrusion technique from polymer science to pharmaceutical applications. Here the polymer PEG provides a useful matrix for the dispersion of hydrophobic drugs in the solid state because its semi-crystalline nature exhibits properties that are useful in drug formulation. The amorphous content may make it possible to incorporate other components into the structure. However, eventually, a hydrophobic additive will usually crystallise to form a separate, dispersed phase, whereby a solid dispersion is formed.

An interesting idea could be to use this phase separation to create a pre-dispersed lipid system, i.e., a solid dispersion that will transform into a lipid dispersion upon dissolution. Dispersing a lipid in the dry state might prevent leakage of the incorporated drugs during preparation, which is a problem when the process is carried out in water. Using MO as a model lipid could be of additional interest here, since it gives rise to a cubic phase upon contact with water and consequently may be able to accommodate a large variety of drugs. MO dispersed in water, a type of cubosome, has shown great drug carrier abilities.

Within polymer related research of polymers SAXS is a widely used technique for the study of solid semi-crystalline polymers. In the context of solid

dispersions, it would be interesting to use this technique to obtain structural information on a nano size scale. In addition, it might give information on the mechanisms by which hydrophobic components are incorporated in PEGs. The MO/PEG system is considered to be suitable for the observation of the general mixing behaviour of a semi-crystalline hydrophilic polymer with a lipid.

## Aims of the thesis

The underlying idea behind this thesis was to study phase formation and transformations in mixed solid systems of a pharmaceutical interest. A special emphasis was put on the usefulness of the AFM and SAXS techniques to characterise materials that could adopt both amorphous and crystalline states.

Two different systems were chosen and specifically the following points were focused on:

1. Amorphous particles of lactose with PVP
  - The possibility of visualising the crystallisation of amorphous spray-dried particles was evaluated using AFM
  - Measuring the kinetics for the crystallisation
  - Characterising the surface composition and crystallisation as a function of added PVP molecular weight and concentration.
2. Solid state mixture of PEG and MO
  - Characterising MO/PEG solid dispersions formed by co-melting and solidification
  - Visualising solid dispersion domains by AFM
  - Studying the lamellar packing of PEG and MO in the solid dispersion by the use of SAXS and DSC
  - Studying the effect of the molecular weight of PEG and of added cyclosporine and desmopressin on the phase formation of the solid dispersion
  - Studying the incorporation of these peptides into the structure

# Preparation and characterisation of the systems

## Lactose / PVP

### Preparation of particles

Lactose and composite lactose/PVP particles were prepared by spray-drying (Niro Atomizer A/S, Denmark) water solutions of the components. The composite particles comprised of 5 and 25% PVP with two different molecular weights, either PVP K17 (10,000 g/mol) or PVP K90 (1,100,000 g/mol). Particles of a certain size (5–15  $\mu\text{m}$ ), known as the size fraction, were selected by air classification (Alpine 100 MZR, Alpine AG, Germany) and stored at room temperature in a desiccator with  $\text{P}_2\text{O}_5$  (0% RH). X-ray diffraction analysis indicated that the particles were completely amorphous (Diffractor D5000 Siemens, Germany).

### AFM

AFM imaging was performed at 25 °C using a PicoSPM (Molecular Imaging, AZ, USA) in acoustic AC mode. During scanning the particles were dispersed on a glass slide, which made imaging of single particles possible. The RH was adjusted by mixing dry nitrogen gas with humidified nitrogen gas (bubbled through deionised water) before introducing the gas mixture into a hermetically sealed chamber in which the sample was scanned. The RH was monitored while scanning, using a calibrated hygrometer.

For kinetic measurements, single particles were scanned repeatedly at low RH, whereupon the RH was quickly increased to induce the crystallisation. The time point at which the rise in RH was brought about was taken to be the start of the experiment. The images were then obtained, with each scan lasting for 10 minutes, until no more observable changes could be detected.

## AFM image analysis

The average deviation (AD, also known as rugosity) was determined from the topography images using the software provided with the AFM equipment (Visual SPM, Molecular Imaging, AZ, USA). The rugosity is defined by

$$AD = \frac{\sum_{i=1}^n |y_i - \bar{y}|}{n} \quad (6)$$

where  $n$  is the number of data points and  $y_i$  the height coordinate of the  $i$ th data point and  $\bar{y}$  the average height coordinate of all data points. The fraction of the surface that had been crystallised, called the ‘fraction of surface crystallised’ ( $\alpha$ ) was calculated using:

$$AD_{av} = \alpha_L AD_{cr} + (1 - \alpha_L) AD_{am} \quad (7)$$

where  $AD_{am}$  was measured from the topography images of the uncrystallised smooth particle surface and  $AD_{cr}$  determined from images of the surface after crystallisation. These values were plotted *versus* time for each particle studied and the crystallisation rate constant determined by fitting the JMAK-equation (Equation 5) to the plot.

## ESCA

The ESCA analysis was made with a Scienta ESCA-300 instrument on tablets ( $\emptyset$  13 mm) prepared by compaction (<10 MPa) of approximately 200 mg of spray-dried particles. The surface composition was calculated from the relative amount of the element present determined by measuring the area under the oxygen 1s and nitrogen 1s lines in the ESCA spectra and from the molecular formulas of the components.

## MO / PEG

### Preparation of solid mixtures

Solid mixtures of PEG with different amounts of MO and peptide were prepared by melting the components at 70 °C (MO/PEG 4000 samples) or 120 °C (MO/PEG 1500, 4000 and 8000, with and without peptides) in glass

ampoules for 20 min during intermittent vortexing. Solidification subsequently occurred upon storage at room temperature (23 °C). The samples were stored for at least six weeks and a light grinding of the samples in the glass ampoules to produce a crude powder was done before using it for DSC and X-ray diffraction analysis.

## DSC

DSC was performed in dry nitrogen gas using a Seiko DSC 220 differential scanning calorimeter SSC/5200H (Seiko, Japan). The analysis of each sample (2-4 mg) was started after 5 minutes of cooling at 0 °C, by heating at a rate of 5 °C/min until a temperature of 150 °C was reached and the sample had been completely melted. The heat of melting per gram sample ( $\Delta H_m$ ) was determined by measuring the area of the peak representing the melting of each component. The weight fraction of the MO phase was determined from

$$X_{MO\text{phase}} = \frac{\Delta H_m^{MO, \text{sample}}}{\Delta H_m^{MO, \text{pure}}} \quad (8)$$

## SAXS

SAXS experiments were performed with a Kratky camera (Hecus X-ray systems, Graz, Austria) for 3 h in a vacuum using Cu K $\alpha$  X-rays (of wavelength 1.542 Å) provided by an X-ray generator (Philips, PW 1830/40, The Netherlands). Diffracted X-ray was detected with a linear position-sensitive detector (MBraun, Garching, Germany).

The experimental SAXS diffractogram allowed the one-dimensional correlation function to be calculated from the scattering originating from the PEG lamellar structure. This yielded values for the  $L$ ,  $l_c$  and  $l_a$  of the lamellar structure of PEG within the samples. For the MO/PEG 4000 a mere truncation of the diffractogram was enough to eliminate the influence of the MO diffraction peak on the calculation of the one dimensional correlation function of the PEG structure. An alternative way to eliminate the scattering from MO was implemented in order to reduce problems with overlapping peaks in the diffractograms. A theoretical peak, of the type known as split Pearson VII, was fitted to the MO diffraction peak and subtracted from the diffraction pattern before the correlation function was calculated.

## WAXS

WAXS was performed on the MO/PEG 4000 samples with a Diffractor D5000 (Siemens, Germany) equipped with a scintillation detector using Cu- $K_{\alpha}$  radiation, 45 kV and 40 mA. Bragg-Brentano focusing geometry was used and each sample measured twice to ensure that the X-rays did not influence the sample.

## AFM

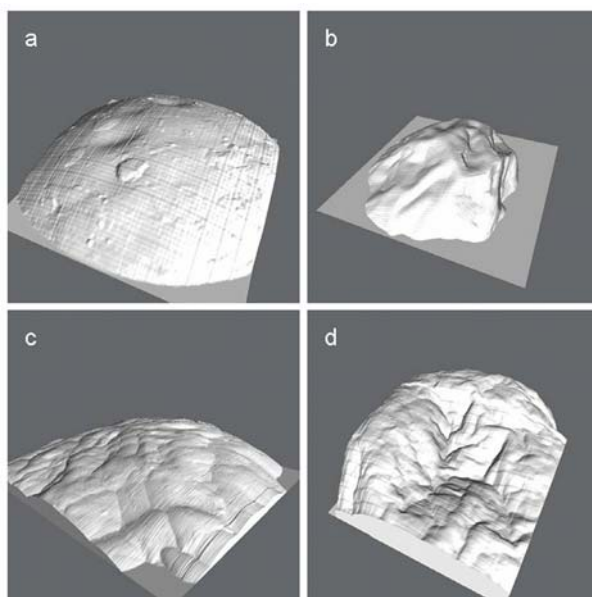
AFM imaging was performed by using a PicoSPM (Molecular Imaging, AZ, USA) in the acoustic AC mode. Various temperatures were tried in the range 10 to 50 °C to find a detectable contrast between the MO and PEG domains. Scanning was done on differently produced surfaces of the samples, i.e., the ones formed in contact air, glass or cleaved mica and surfaces formed either by cutting or by grinding the samples.

# Results and discussion

## Lactose / PVP

### Particle surface composition and topography

AFM and SEM revealed that the spray-dried lactose particles were relatively spherical displaying only minor deformations. The particles containing PVP had a more uneven surface topography. AFM images in Figure 14 shows typical images of the different particles. It was apparent that particles with the highest PVP content (25%) showed the highest degree of surface folding. Since PVP increases the viscosity of water solutions, it can be hypothesised that the contraction taking place in the spray-drying process was affected by the presence of PVP and may have contributed to the folded surface topography of the particles.



*Figure 14.* 3D AFM topography images of dry spray-dried composite particles consisting of lactose and PVP. The compositions of the different particles are (a) pure lactose, (b) 25% PVP K17, (c) 5% PVP K90 and (d) 25% PVP K90. The length of the side of the 3D section is 10 $\mu$ m.

The surface layer (~10 nm thick) of the spray-dried composite particles was richer in PVP than in the remainder of the particle. The composition of the particle surfaces is presented in Table 1. The PVP surface enrichment was further confirmed by measurements at a 10° grazing angle, which showed that the PVP content was highest in the topmost layer.

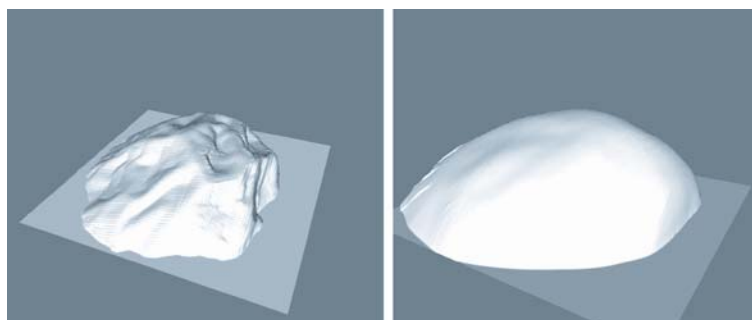
*Table 1.* Surface composition, the RH interval where smoothening of the particle surface was observed and  $T_g$  measured on dry powders consisting of the different spray-dried particles.

Particle composition	Surface composition of particle (wt % PVP)	Observed surface smoothening at RH (%)	$T_g$ (°C) of dry powder (from ref 28)
Lactose	—	44 – 48	116.8
5% PVP K17	31	52 – 56	117.2
5% PVP K90	26	51 – 56	117.5
25% PVP K17	56	57 – 60	117.7
25% PVP K90	51	60 – 66	117.8

The total amount of PVP in the sample seemed to be more important for the surface composition than the molecular weight of the polymer. As it has been shown that PVP adsorbs to the water-air interface<sup>15</sup>, it seems likely that the PVP was enriched at the droplet surface during the spray-drying, before a surface crust was formed, which could possibly explain the higher surface content of PVP K17 compared to PVP K90. The hundred times smaller PVP K17 can be transported faster in a water solution and hence would be likely to reach the surface to a higher extent than PVP K90 during the short period that the liquid droplet exists ( $\ll 1$  s).<sup>23</sup>

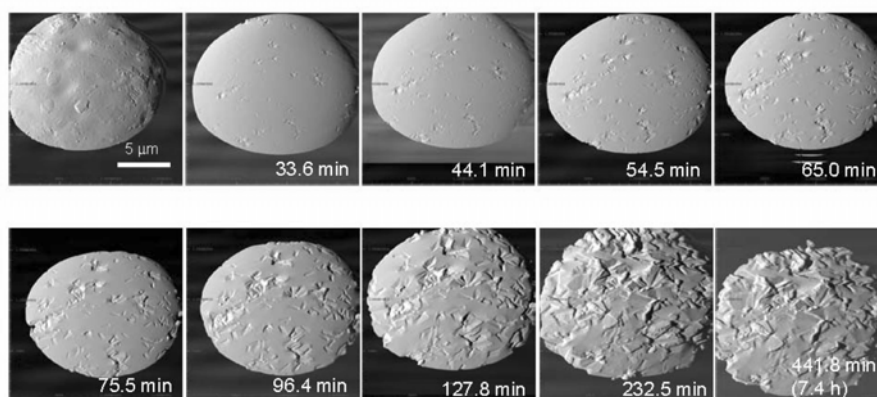
### Response to increased relative humidity

When particles were exposed to higher RHs ( $> 40\%$ ), a gradual swelling and smoothening of their surface occurred. This transition was detected within an RH interval, which average value was dependent on the particle composition. These RH intervals are shown for the different types of particles in Table 1. A typical image of a particle before and after smoothening is shown in figure 15. Particles containing PVP had to be exposed to a higher RH than those of pure lactose before the smoothening of the surface occurred.



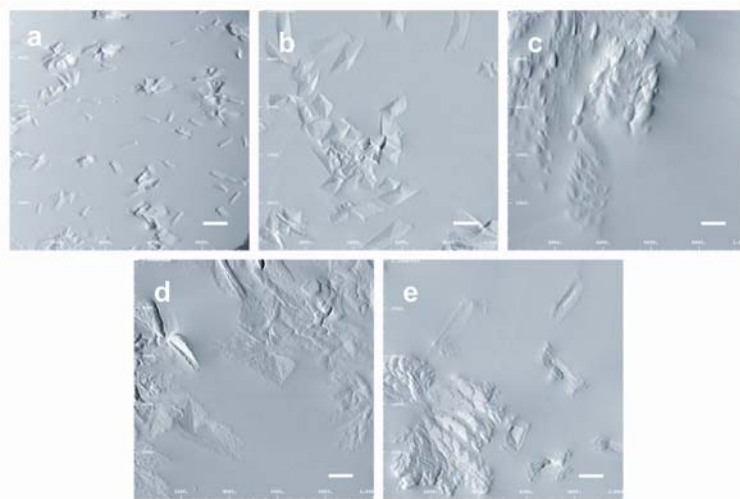
*Figure 15.* A typical 3D AFM topography image of a particle before and after smoothening due to increased RH.

The smoothening of the lactose particle surface occurred at an RH where the lactose had incorporated water to such an extent that the  $T_g$  was as low as the ambient temperature. Hence it is likely that the smoothening of the surface was enabled by the increased molecular mobility associated with the transition from the glassy state to a super-cooled liquid (i.e. the rubbery state). The surfaces of the PVP containing particle, on the other hand, were probably less flexible owing to their high PVP content and, consequently, required a higher RH to be smoothen.



*Figure 16.* AFM amplitude images of a single amorphous lactose particle crystallising at 50% relative humidity. The time given for each image represents the time from when the RH was increased. First image was scanned before start of experiment.

The particles eventually crystallised at a rate that was dependent on the RH and the amount and molecular weight of the PVP. In figure 16 it is shown how small crystallites appeared on the smooth surface and grew continuously until the whole surface was covered. The crystallites of pure lactose could be described as rectangular shaped depressions which were more or less distorted by the presence of 5% PVP, whereas the surface of the particles that contained 25% PVP was covered with humps that grew over time and were typically arranged in lines or clusters at different locations (see figure 17).



*Figure 17.* AFM amplitude images of crystallising lactose/PVP particles. (a) Pure lactose, (b) 5% PVP K17, (c) 25% PVP K17, (d) 5% PVP K90 and (e) 25% PVP K90. White bars in images indicate 1 $\mu$ m.

### Quantification of crystallisation

In order to quantitatively analyse crystal growth, the possibility of measuring the growth rate of single crystals on the surface was examined. In the case of pure lactose it was possible to measure the length and width for a number of crystals ( $n = 4$ ) on the same particle at different time intervals and plot the results as a function of time as shown in figure 18. All quantitative measurements were made on flattened topographical images of the particle surfaces. The relationships were approximately linear, with an expected deviation from linearity after approximately 90 minutes, caused by crystals starting to impinge on the space occupied by other crystals around that time. The crystal growth rate was then determined from the initial linear slopes. The average rates of growth were  $14.4 \pm 3.1$  nm/min on the long axis and  $6.9 \pm 2.4$  nm/min across the width of the crystallites of pure lactose at 54 % RH.

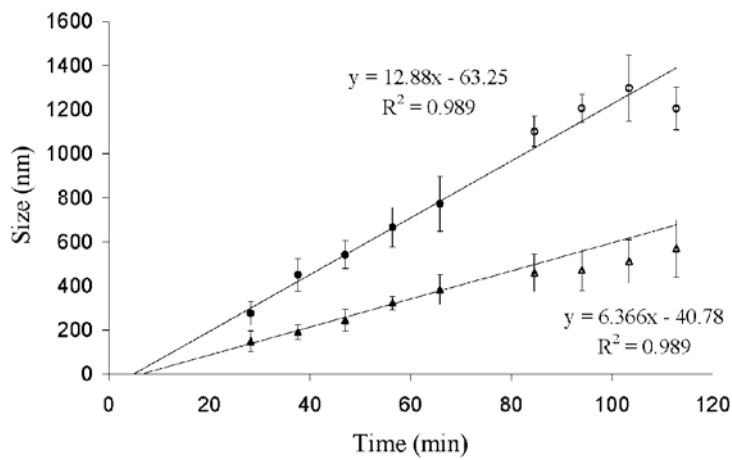


Figure 18. Growth of single rectangular crystals ( $n=4$ ) as a function of time, width (triangles) and length (circles). Linear regression done on solid dots. Mean values. Error bars denote standard deviations.

The property of interest for the present analysis was the degree of surface crystallinity. To be able to measure this property the surface roughness parameter  $AD$  was determined on flattened topographical images. The  $AD$ -values obtained at different time points together with those determined on the amorphous surface ( $AD_{am}$ ) and on the crystalline ( $AD_{cr}$ ) of a particle were used for calculating the fraction of surface crystallised ( $\alpha_L$ ).

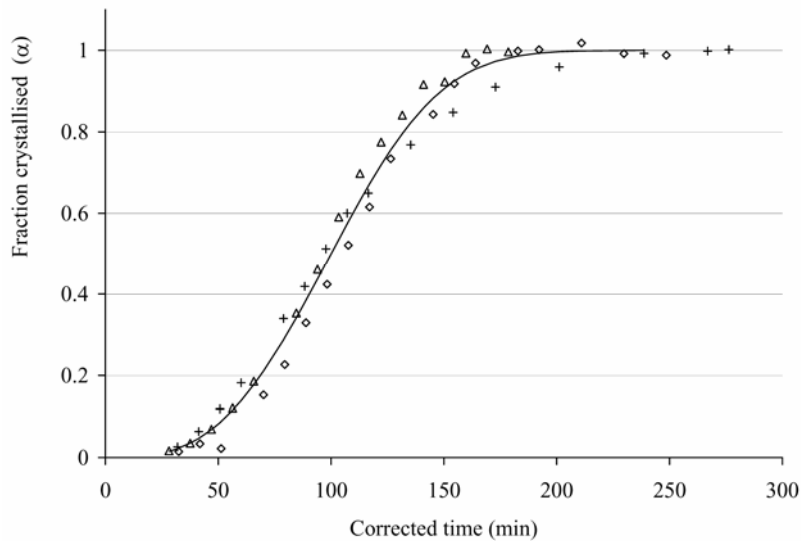


Figure 19. Fraction of crystallised surface as a function of corrected time. Values determined from average deviation data obtained from three separate runs. Solid line shows best fit to the general JMAK equation.

Figure 19 shows  $\alpha$  for pure lactose plotted as a function of time. The solid curve represents the best fit of the JMAK equation (Equation 5) to the data and as can be seen, a good fit was obtained, from which the rate constant was obtained for the crystallisation. This procedure was repeated for particles containing PVP whose crystallisation rate had been measured for a range of RHs. The plot of rate constants as a function of RH can be found in Figure 20.

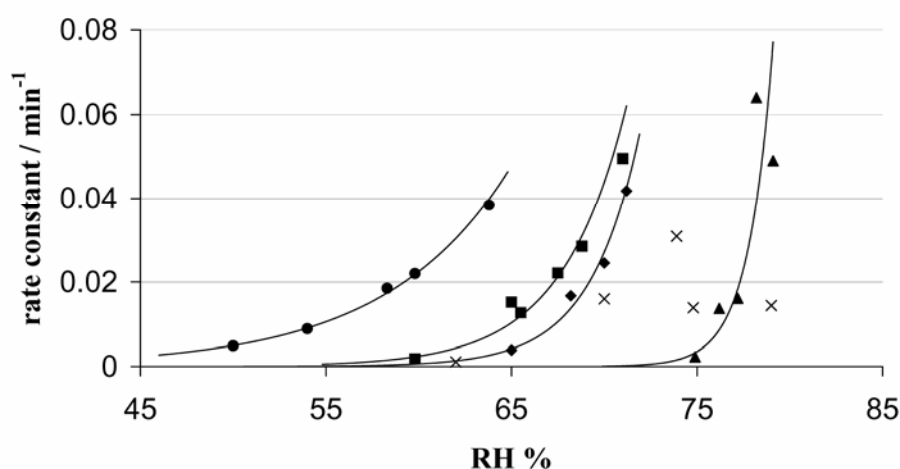


Figure 20. The rate constant of surface associated crystallisation plotted *versus* RH for pure lactose (filled circles) and lactose with 5% PVP K17 (filled squares), 5% PVP K90 (filled diamonds), 25% PVP K17 (x) and 25% PVP K90 (filled triangles). The solid line curves indicate best fit of an exponential function.

The presence of PVP obviously inhibited crystallisation in the way that a higher RH was needed to obtain a crystallisation rate that was measurable at in time-scale of the AFM measurement. The obvious exception here was the set of samples containing 25% PVP K17 where the values were scattered and the dependence on RH was less clear cut. The reason for this was probably an at this time unexplained lack of uniformity in the amount of PVP in the different particles. Alternatively the number of nucleation sites could have been reduced to less than one per particle.

The inhibition that PVP had on the crystallisation rate seemed to be more dependent on the amount of PVP present than on the molecular weight (at 5% PVP). This was unexpected since the molecular weight is usually considered to have a large impact on the ‘time to the crystallisation peak’ as measured by MC on lactose/PVP powder samples.<sup>31</sup> The surface associated

crystallisation studied here may, however, be affected by the surface adsorption that evidently occurs in the spray-dried particles. The indications that the PVP content of PVP K17 is higher than that of PVP K90 at the surface may contribute to a higher inhibition than expected from the molecular weight.

*Table 2.* The parameters obtained from the fit of Equation 9 to the measured rate constants. R is the regression coefficient.

Particles	$k_0 / \text{min}^{-1}$	$b / \text{RH}^{-1}$	R
Lactose	$2.61 \cdot 10^{-6}$	0.151	0.9989
5% PVP K17	$7.07 \cdot 10^{-11}$	0.289	0.9810
5% PVP K90	$1.00 \cdot 10^{-13}$	0.376	0.9915
25% PVP K17	—	—	—
25% PVP K90	$9.86 \cdot 10^{-28}$	0.754	0.9406

The higher exponential increase in the rate constant was analysed by fitting an exponential equation

$$k = k_0 e^{bRH} \quad (9)$$

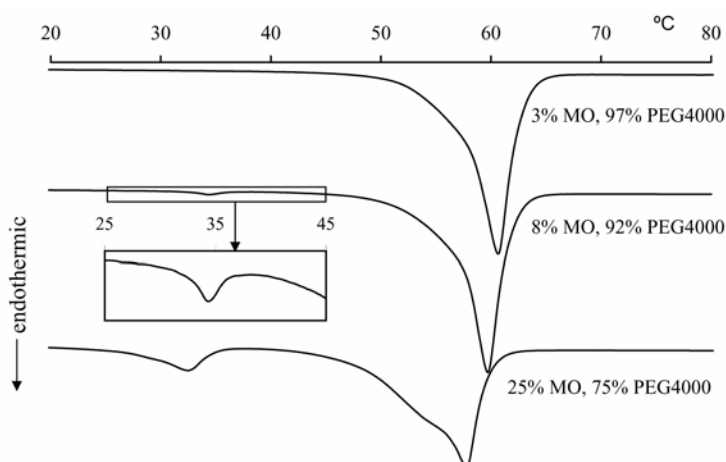
The obtained  $k_0$  and  $b$  values from the fit of Equation 9 can be found in Table 2. The  $k_0$ -values correspond to the value of  $k$  extrapolated to where the RH is equal to zero. These values reflect the relative stability i.e. the shift of the RH where crystallisation reaches a detectable level as well as the stability at dry conditions. The exponential raise of the rate constants, i.e. the  $b$ -values, increased as the PVP content increases. This phenomenon can be interpreted as a higher sensitivity to an increased RH above the point where crystallisation was detected. This shows that the absorption of water into the particles affect the stabilising effect of PVP. It seems that nucleation could be inhibited to a larger extent than the molecular mobility when PVP was present and the moisture content in the particles increased. The RH at which crystallisation appeared was higher in the composite particles but when nucleation eventually occurred the rate of crystallisation was rapid. This emphasises the importance of understanding the mechanisms of stabilization of amorphous composites in terms of interaction between mixed components and as a combination of nucleation and growth.

## MO / PEG

### Phase identification

Unpublished results from earlier studies within the research group indicated that a solidified melt of MO and PEG 4000 formed a solid dispersion where the MO was dispersed as small domains within the structure. This conclusion was drawn from dynamic laser scattering experiments where such a sample had been dissolved in water and the size of the dispersed particles that were released then measured. The size of the domains of MO in water was found to be approximately 200 nm.

DSC was performed on the solid samples which confirmed the expected PEG and MO phase separation upon solidification. Two separate melting peaks were visible in the DSC melting thermogram (see Figure 21), approximately overlapping the melting peaks of the pure components.



*Figure 21.* DSC thermograms of MO/PEG 4000 solid state mixtures. The lower endothermic peak shows the melting of MO and the higher the melting of PEG.

The melting point of MO was nearly constant over all compositions of the MO/PEG 4000 mixture while, the melting point associated with PEG decreased gradually as the amount MO increased (Figure 22). The MO/PEG 1500 and MO/PEG 8000 mixtures exhibited the same melting characteristics for the compositions studied (0-25 and 0-45 % MO, respectively) so it could be concluded that the MO/PEG mixture behaved like a monotectic system.

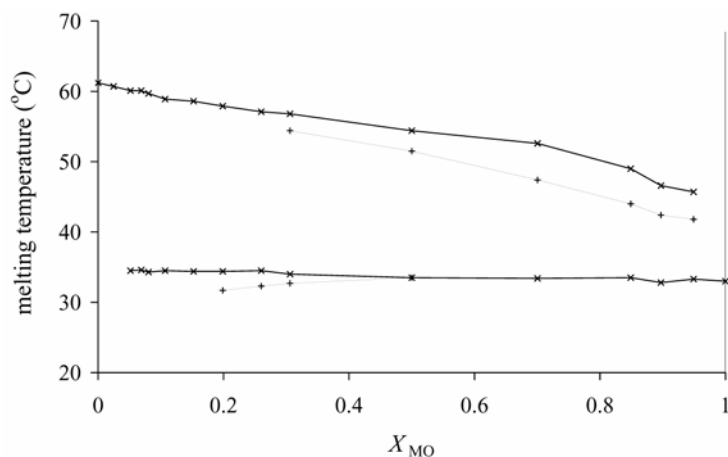


Figure 22. Melting temperatures determined from peak maxima in DSC thermograms. The upper curve (liquidus line) indicates the melting of PEG and the lower one (solidus line) the melting of MO. The upper grey line shows the once folded PEG melting temperature while the lower one represents the melting of the second MO phase.

MO displayed a double melting peak for the mixtures of MO/PEG 4000 and MO/PEG 1500, with the first peak appearing when MO was present in 5 wt % in both types of mixtures and the second MO phase detectable at 10 and 15 wt % MO, respectively.

A WAXS diffraction pattern of the MO/PEG 4000 samples showed diffraction peaks originating from the  $\beta$ -form of MO in the samples with 20 and 30 % MO. As WAXS indicated no other MO polymorphs than the  $\beta$ -form, the higher melting point for the second form can be explained by a different incorporation of this form into the PEG structure

## Incorporation of MO

Figure 23 shows the amount MO phase that was formed when the total fraction of MO ( $X_{MO}$ ) was increased in the mixtures comprised of PEG of the different molecular weights (1,500, 4,000 and 8,000 g/mol). Under the assumption that all MO which could not be detected within the separate MO phase was incorporated into the PEG-rich phase, the DSC-data shows that the PEG-rich phase in the different PEGs contained approximately 20, 8,5 and 5 % MO, respectively. By analysing the shape of the PEG melting peak in the DSC thermograms it was evident that the folding of PEG increased with MO content.

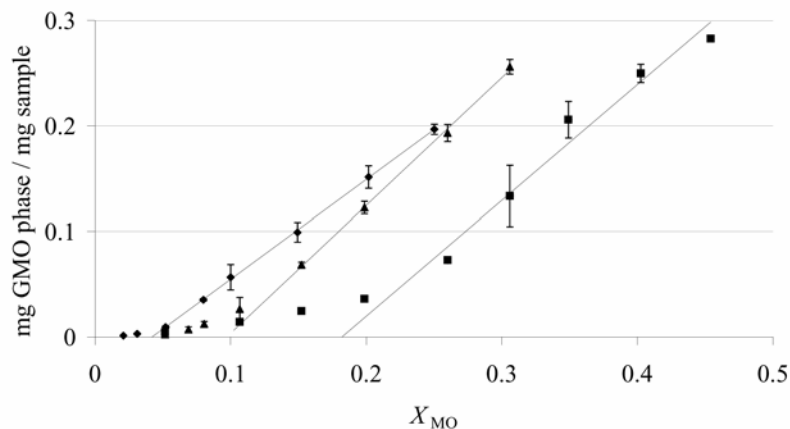


Figure 23. Amount MO phase formed at different compositions. The average molecular weights of the PEGs in the mixtures are 8000 (diamonds), 4000 (triangles) and 1500 (squares). Error bars indicated standard deviation and straight lines best fit to linear region of the plots.

From the SAXS data analysis conducted for Paper III it was evident that the amorphous length of the PEG lamellar structure,  $l_a$ , increased in parallel with the increasing amount of MO that was intercalated as determined by DSC. In Figure 24 the change in dimensions of the lamellar structure are changing as MO content increases is shown. As WAXS data detected no affect on the crystalline packing of the PEG, it could be safely assumed that the MO was not intercalated into the crystalline phase. Thus it was concluded that the MO is incorporated in the amorphous parts of the lamellar structure.

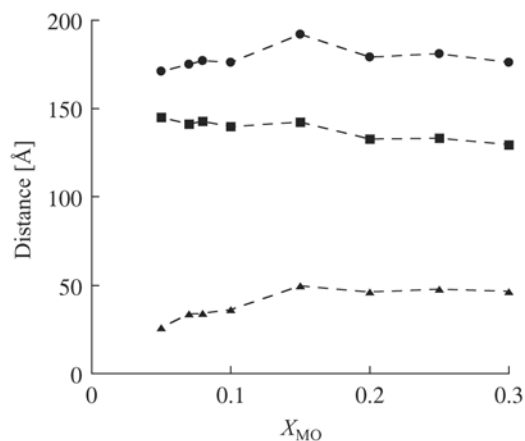


Figure 24. Obtained L (circles),  $l_c$  (squares) and  $l_a$  (triangles) for the lamellar structure of PEG 4000 in mixtures with MO.  $X_{MO}$  is the weight fraction of MO in the mixture.

A 10% reduction (typically) in all calculated distances ( $L$ ,  $l_a$  and  $l_c$ ) was evident when comparing the results obtained from the analysing procedure described in Paper IV, compared with the one used in Paper III. However,

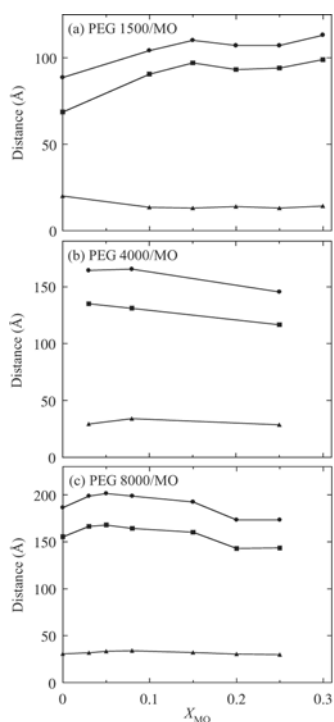


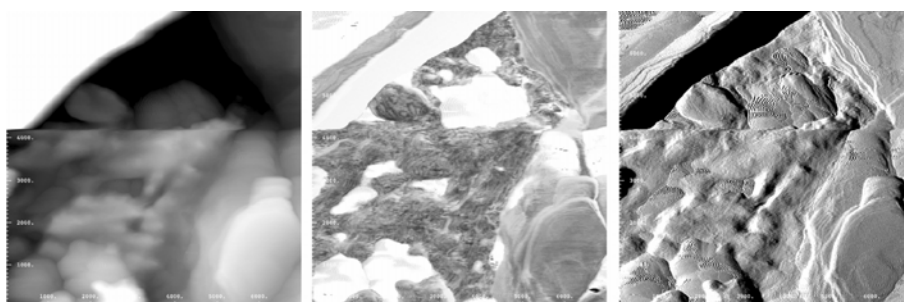
Figure 25.  $L$  (circles),  $l_c$  (squares) and  $l_a$  (triangles) of the lamellar structure of PEG in mixtures with PEGs of different molecular weights.  $X_{MO}$  is the weight fraction of MO in the mixture.

comparisons of distances for the same types of samples analysed by the same method were considered to be appropriate. The crystalline distance ( $l_c$ ) decreased for both PEG 4000 and 8000 with increasing wt % MO, which indicates that the degree of folding had increased (see Figure 25). PEG 1500 unfolded when mixed with MO so that  $l_c$  became equal to the length of an extended polymer helix. Hence, it seemed to be necessary for folding to take place for MO to be incorporated in PEG 4000 and 8000. PEG 1500 on the other hand is more unstable in the folded forms. A simple estimation based on the dimensions of the PEG unit cell<sup>100</sup> and lamellar structure shows that PEG 1500 has greater volume in between the extended crystal lamellae in which to accommodate MO than the extended PEGs of higher molecular weights. This may also contribute to the larger observed capacity to intercalate MO.

The PEG unfolding over the first week of storage occurred simultaneously with the appearance of a second MO phase that was detected with DSC in the mixtures of MO/PEG 1500 and MO/PEG 4000. It is likely that a second MO phase forms upon expulsion of MO from the PEG structure and that this is a consequence of the unfolding process. There was no second form detected in the mixture comprising of MO and PEG 8000. From this observation it was hypothesised PEG 8000 intercalated less MO early in the solidification process, which led to that less MO could be expelled upon unfolding. This can be argued by considering the driving force for phase separation, which usually is larger for polymers of higher molecular weight, because there is less entropic loss than for the corresponding low molecular weight polymers. In addition, PEG 8000 is more stable in the folded state which was confirmed to be true with SAXS data in this study. This hypothesis could explain why PEG 1500 has a large amount of incorporated MO even if there is an expulsion of the lipid upon the unfolding of the PEG helices.

## AFM imaging

The imaging performed on MO/PEG samples did not give reproducible results. Although some images were of good quality the variability made it impossible to obtain systematic results. It is likely that the soft MO domains were the source of the inconsistencies since the problems increased with increasing MO content. A smearing of the images was indicative of MO having become attached to the tip. In Figure 26 an example of a good image is shown, displaying possible domains.



*Figure 26.* AFM topography, phase and amplitude images (from left to right) of the solid dispersion of MO in PEG 4000. Different domains are clearly visible in the phase image (middle) where the white area almost certainly shows separate MO phases.

## Incorporation of peptides

In this study we were also interested in how a potential drug would incorporate into the structure. A hydrophilic (desmopressin) and hydrophobic (cyclosporine) peptide was chosen for this purpose and one or the other of these peptides was added to an amount that corresponds to 20 wt % of the MO wt %. At these concentrations no significant effect could be detected on the measured parameters using DSC and SAXS. The only noteworthy observation was a 10Å increase in  $l_c$  for all the samples containing desmopressin. The reason for this is not clear. Problems with dissolving the peptides in the melted mixture of PEG and MO may have contributed to the absence of positive results.

## Summary and Conclusions

In this thesis the transformation of amorphous lactose/PVP particles into the crystalline state and the formation of phases in solid dispersions of MO in PEG were studied. The first of these systems can be described as a solid solution which was induced to phase separate by exposure to humidity. The second one, essentially a solid dispersion, phase separated upon solidification from a co-melt. Although these systems are quite different, they have fundamental biopharmaceutical, physical and chemical features in common. In particular, both the amount and the molecular weight of the polymers in the systems were important for the phase formation and transformations studied.

### Moisture induced transformations of amorphous particles

Amorphous materials could have significant benefits for the formulation of pharmaceuticals if the problems with the physical stability over time were to be solved. The propensity for a material to crystallise is usually studied using powder samples by methods such as DSC, microcalorimetry and gravimetry but these techniques restrict one to the use of powder samples. In contrast, AFM is a technique that can visualise the surface of single micro-sized particles. In the first part of this thesis, this ability was utilised to map the surface topography of spray-dried particles when dry and whilst absorbing water from humid nitrogen gas. The plasticizing effect of the absorbed water, as the RH increased, was manifested by a smoothening and swelling of the particles that took place within an RH interval associated with an increased molecular mobility of the system. A further increase in the RH induced re-crystallisation of the particles which could be monitored by the AFM instrument. In contrast to the traditional methods used, AFM revealed that the crystallisation was a continuous process on the scale of individual particles and could be described in terms of nucleation and crystal growth.

The crystallisation of spray-dried lactose particles was successfully quantified through the surface roughness parameter 'average deviation', which enabled the fraction of the surface of the particles that had been crystallised to be determined as a function of time. This procedure opened up the possibility of analysing the kinetics of crystallisation with the JMAK equation.

It is known that addition of a polymer increases the physical stability of an amorphous solid. The way in which PVP is incorporated into a lactose particle upon spray-drying and whether this is important for the stability had not been studied previously. AFM analysis and ESCA, used in conjunction, provided a means of studying the particle structure and of examining the stabilising effect of the polymer on a single particle level. The PVP was enriched at the surface of the composite particles and its presence clearly inhibited the moisture-induced crystallisation, which had been anticipated. However, in contrast to the results obtained from earlier microcalorimetry measurements, the effect of the molecular weight was small, which could be a consequence of the AFM images examining the surface associated crystallisation and not being able to detect the processes occurring inside the particles, as well as an effect of the high PVP content in the surface region ( $\sim 10\text{\AA}$  depth).

Particles containing PVP were more sensitive to increasing humidities above the RH at which crystallisation was first detected for the type of particle under investigation. This shows that the absorption of water into the particles affects the stabilisation induced by the incorporation of PVP. Such destabilisation emphasises the importance of understanding the mechanisms behind the increased stability of amorphous composites, in terms of interactions between mixed components and inhibition of both nucleation and growth. It also implies that the effect of addition of PVP observed at high humidity cannot be readily extrapolated to dry or low moisture conditions.

Through the use of AFM, single particles were studied under well controlled humidity and temperature conditions without being affected by other crystallising particles. This enables systematic investigations to be made of how one single particle responds to and crystallises upon changes in RH, which is important for understanding the crystallisation of a collection of particles as is found in a power sample or tablet. In addition, the effect of PVP on spray-dried particles could be studied, which was of particular interest because it shows the importance of the way in which a polymer is incorporated into the particles for the stability introduced by the polymer. AFM is able to indicate at a very early stage when small amounts of crystalline structures are present on a particle's surface and may, therefore, be a valuable tool for the characterisation of pharmaceutical solids.

## Phase formation in MO / PEG solid mixtures

Solid lipids dispersed into nano-sized particles have a great potential for use as drug delivery vehicles. The production of these particles is usually carried out in an aqueous media by applying large forces to the lipid to break it up into smaller units (e.g., by high pressure homogenisation, high speed stirring or ultra sonication). Surfactants are often used to decrease the particle size; however, this is bound to impose toxicological problems. In last part of this thesis an alternative approach was introduced for dispersing solid lipids, where the inherent phase separation properties of a lipid (MO) and hydrophilic polymer (PEG) were utilized. In addition, this solid mixture was useful because it provided information on how the phases of solid dispersions and solutions formed.

A melt of MO and PEG appeared as one single phase, which phase separated upon cooling. Preliminary data have shown that there is a formation of nano-sized MO domains within the solid mixture upon solidification in room temperature. In this thesis, the structure of the solid mixture of PEG and MO was further investigated using DSC, WAXS and SAXS. Pure MO phase and a PEG-rich phase were formed upon solidification, as anticipated. The amount of MO that was incorporated into the PEG-rich phase depended on the molecular weight of the polymer and was achieved by intercalation of MO molecules into the amorphous domains of the PEG lamellar structure. The PEG with the lowest molecular weight (PEG 1500) incorporated the largest amount of MO. It was hypothesised that a PEG with a higher molecular weight would phase separate to a greater extent during the initial solidification, thereby leaving less MO in the PEG-rich phase, compared to the amount in the low molecular weight PEG. Our results revealed that MO could crystallise into two types of phases, one already present shortly after solidification, and the other formed as more MO was expelled from the PEG-rich phase upon the unfolding of PEG molecules. However, the latter form was not detected in the PEG 8000 mixture which, according to the hypothesis, was due to a low MO content in the initial PEG-rich phase, accompanied by a lower propensity for this molecular weight to unfold. The amount of MO consequently expelled from the PEG 8000 structure was below the detection limit of the methods used.

The incorporation of drugs into lipid particles is notoriously difficult; nowadays it is considered that these problems are best overcome by preventing the lipid particle from completely crystallising to its most stable form, i.e. the  $\beta$ -form. A crystallisation of the lipid is generally considered to be accompanied with an expulsion of the drug from the lipid phase. The formation of lipid domains within the solid PEG did not seem to prevent the formation

of the most stable form of MO. Furthermore, even if two forms are present in the mixture, it is plausible that both are of the  $\beta$ -form, differing only in the way they are incorporated into the solid structure of the mixture. The addition of peptides did not impose detectable changes on the structure of either the MO phase or the PEG-rich phase.

The introduction of these procedures in the analysis has shown that solid mixtures involving PEGs can be used to disperse lipids. Incorporation of peptides into the structure has to be further evaluated and AFM was not able to visualise the domains formed. However it is evident that SAXS could be a tool of use for obtaining information on the degree of folding within a mixture comprised of semi-crystalline polymers when lipid components are to be incorporated into its structure. This opens up new possibilities for the study and control of the formation of solid dispersions, in particular for the production of finely dispersed solid lipids.

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## References

1. Hilden LR, Morris KR 2004. Physics of amorphous solids. *Journal of Pharmaceutical Sciences* 93(1):3-12.
2. Champion D, Le Meste M, Simatos D 2000. Towards an improved understanding of glass transition and relaxations in foods: molecular mobility in the glass transition range. *Trends Food Science and Technology* 11(2):41-55.
3. Craig DQM, Royall PG, Kett VL, Hopton ML 1999. The relevance of the amorphous state to pharmaceutical dosage forms: glassy drugs and freeze dried systems. *International Journal of Pharmaceutics* 179(2):179-207.
4. Hancock BC, Zograf G 1997. Characteristics and significance of the amorphous state in pharmaceutical systems. *Journal of Pharmaceutical Sciences* 86(1):1-12.
5. Craig DQM 1990. Polyethylene Glycols and Drug Release. *Drug Development and Industrial Pharmaceutics* 16(17):2501-2526.
6. Hancock BC, Zograf G 1993. The Use of Solution Theories for Predicting Water-Vapor Absorption by Amorphous Pharmaceutical Solids - a Test of the Flory-Huggins and Vrentas Models. *Pharmaceutical Research* 10(9):1262-1267.
7. Zhang J, Zograf G 2001. Water vapor absorption into amorphous sucrose-poly(vinyl pyrrolidone) and trehalose-poly(vinyl pyrrolidone) mixtures. *Journal of Pharmaceutical Sciences* 90(9):1375-1385.
8. Ottenhof MA, MacNaughtan W, Farhat IA 2003. FTIR study of state and phase transitions of low moisture sucrose and lactose. *Carbohydrate Research* 338(21):2195-2202.
9. Jolley 1970. The Microstructure of Photographic Gelatin Binders. *Photographic science and engineering* 14(3):169-177.
10. Zhou DL, Schmitt EA, Zhang GG, Law D, Vyazovkin S, Wight CA, Grant DJW 2003. Crystallization kinetics of amorphous nifedipine studied by model-fitting and model-free approaches. *Journal of Pharmaceutical Sciences* 92(9):1779-1792.
11. Kibbe A editor 2000. *Handbook of Pharmaceutical Excipients*. 3rd ed., London: The Pharmaceutical Press.
12. Garnier S, Petit S, Coquerel G 2002. Dehydration mechanism and crystallisation behaviour of lactose. *Journal of Thermal Analysis and Calorimetry* 68(2):489-502.
13. Jouppila K, Kansikas J, Roos YH 1998. Crystallization and X-ray diffraction of crystals formed in water-plasticized amorphous lactose. *Biotechnology Progress* 14(2):347-350.
14. Dincer TD, Parkinson GM, Rohl AL, Ogden MI 1999. Crystallisation of alpha-lactose monohydrate from dimethyl sulfoxide (DMSO) solutions: influence of beta-lactose. *Journal of Crystal Growth* 205(3):368-374.
15. Huang QR, Wang CH 1996. Surface laser light scattering studies of the air/poly(N-vinyl-2-pyrrolidone)-water solution interface. *Journal of Chemical Physics* 105(15):6546-6552.

16. Buckley CP, Kovacs AJ 1976. Melting Behavior of Low-Molecular Weight Poly (Ethylene-Oxide) Fractions .2. Folded Chain Crystals. *Colloid and Polymer Science* 254(8):695-715.
17. Shah JC, Sadhale Y, Chilukuri DM 2001. Cubic phase gels as drug delivery systems. *Adv Drug Delivery Reviews* 47(2-3):229-250.
18. Larsson K. 1994. *Lipids - Molecular organization, physical functions and technical applications.* ed., Dundee, Scotland: The Oily Press LTD.
19. Malkin T 1954. The polymorphism of glycerides. *Progress in the Chemistry of Fats and other Lipids* 2:1-50.
20. Meinzer A, Mueller E, Vonderscher J 1995. Microemulsions- A Suitable Galenical Approach for the Absorption Enhancement of Low Soluble Compounds? *Journées Galéniques* 88:21-26.
21. Buchwald P, Bodor N 1998. Octanol-water partition of nonzwitterionic peptides: Predictive power of a molecular size-based model. *Proteins-Structure Function and Genetics* 30(1):86-99.
22. Lundin PDP, Bojrup M, LjusbergWahren H, Westrom BR, Lundin S 1997. Enhancing effects of monohexanoin and two other medium-chain glyceride vehicles on intestinal absorption of desmopressin (dDAVP). *J Pharmacol Exp Ther* 282(2):585-590.
23. Masters K. 1991. *Spray Drying Handbook.* 5 ed., Harlow, England: Longman Scientific & Technical.
24. Elversson J, Millqvist-Fureby A, Alderborn G, Elofsson U 2003. Droplet and particle size relationship and shell thickness of inhalable lactose particles during spray drying. *Journal of Pharmaceutical Sciences* 92(4):900-910.
25. Leuner C, Dressman J 2000. Improving drug solubility for oral delivery using solid dispersions. *European Journal of Pharmaceutics and Biopharmaceutics* 50(1):47-60.
26. Sethia S, Squillante E 2003. Solid dispersions: Revival with greater possibilities and applications in oral drug delivery. *Critical Reviews in Therapeutic Drug Carrier Systems* 20(2-3):215-247.
27. Gordon M, Taylor JS 1952. Ideal Copolymers and the Second-Order Transitions of Synthetic Rubbers. I. Non-Crystalline Copolymers. *Journal of Applied Chemistry* 2:493-500.
28. Shamblin SL, Huang EY, Zografi G 1996. The effects of co-lyophilized polymeric additives on the glass transition temperature and crystallization of amorphous sucrose. *Journal of Thermal Analysis* 47(5):1567-1579.
29. Imamura K, Fukushima A, Sakaura K, Sugita T, Sakiyama T, Nakanishi K 2002. Water sorption and glass transition behaviors of freeze-dried sucrose-dextran mixtures. *Journal of Pharmaceutical Sciences* 91(10):2175-2181.
30. Shamblin SL, Taylor LS, Zografi G 1998. Mixing behavior of colyophilized binary systems. *Journal of Pharmaceutical Sciences* 87(6):694-701.
31. Berggren J, Alderborn G 2003. Effect of polymer content and molecular weight on the morphology and heat- and moisture-induced transformations of spray-dried composite particles of amorphous lactose and poly(vinylpyrrolidone). *Pharmaceutical Research* 20(7):1039-1046.
32. Takeuchi H, Yasuji T, Yamamoto H, Kawashima Y 2000. Temperature-induced crystallization and compactibility of spray dried composite particles composed of amorphous lactose and various types of water-soluble polymer. *Chemical and Pharmaceutical Bulletin* 48(4):585-588.

33. Matsumoto T, Zografi G 1999. Physical properties of solid molecular dispersions of indomethacin with poly(vinylpyrrolidone) and poly(vinylpyrrolidone-co-vinylacetate) in relation to indomethacin crystallization. *Pharmaceutical Research* 16(11):1722-1728.
34. Crowley KJ, Zografi G 2003. The effect of low concentrations of molecularly dispersed poly(vinylpyrrolidone) on indomethacin crystallization from the amorphous state. *Pharmaceutical Research* 20(9):1417-1422.
35. Aso Y, Yoshioka S, Kojima S 2004. Molecular mobility-based estimation of the crystallization rates of amorphous nifedipine and phenobarbital in poly(vinylpyrrolidone) solid dispersions. *Journal of Pharmaceutical Sciences* 93(2):384-391.
36. Corrigan DO, Healy AM, Corrigan OI 2002. The effect of spray drying solutions of polyethylene glycol (PEG) and lactose/PEG on their physicochemical properties. *International Journal of Pharmaceutics* 235(1-2):193-205.
37. Briggner LE, Buckton G, Bystrom K, Darcy P 1994. The Use of Isothermal Microcalorimetry in the Study of Changes in Crystallinity Induced During the Processing of Powders. *International Journal of Pharmaceutics* 105(2):125-135.
38. Christensen KL, Pedersen GP, Kristensen HG 2002. Physical stability of redispersible dry emulsions containing amorphous sucrose. *European Journal of Pharmaceutics Biopharmaceutics* 53(2):147-153.
39. Surana R, Randall L, Pyne A, Vemuri NM, Suryanarayanan R 2003. Determination of glass transition temperature and in situ study of the plasticizing effect of water by inverse gas chromatography. *Pharmaceutical Research* 20(10):1647-1654.
40. Chidavaenzi OC, Buckton G, Koosha F 2001. The effect of co-spray drying with polyethylene glycol 4000 on the crystallinity and physical form of lactose. *International Journal of Pharmaceutics* 216(1-2):43-49.
41. Hancock BC, Shamblin SL, Zografi G 1995. Molecular Mobility of Amorphous Pharmaceutical Solids Below Their Glass-Transition Temperatures. *Pharmaceutical Research* 12(6):799-806.
42. Di Martino P, Joiris E, Gobetto R, Masic A, Palmieri GF, Martelli S 2004. Ketoprofen-poly(vinylpyrrolidone) physical interaction. *J Cryst Growth* 265(1-2):302-308.
43. Hancock BC, Shamblin SL 2001. Molecular mobility of amorphous pharmaceuticals determined using differential scanning calorimetry. *Thermochimica Acta* 380(2):95-107.
44. Aso Y, Yoshioka S, Kojima S 2001. Explanation of the crystallization rate of amorphous nifedipine and phenobarbital from their molecular mobility as measured by C-13 nuclear magnetic resonance relaxation time and the relaxation time obtained from the heating rate dependence of the glass transition temperature. *Journal of Pharmaceutical Sciences* 90(6):798-806.
45. Andronis V, Zografi G 1998. The molecular mobility of supercooled amorphous indomethacin as a function of temperature and relative humidity. *Pharmaceutical Research* 15(6):835-842.
46. Alie J, Menegotto J, Cardon P, Duplaa H, Caron A, Lacabanne C, Bauer M 2004. Dielectric study of the molecular mobility and the isothermal crystallization kinetics of an amorphous pharmaceutical drug substance. *Journal of Pharmaceutical Sciences* 93(1):218-233.

47. Andronis V, Zografi G 2000. Crystal nucleation and growth of indomethacin polymorphs from the amorphous state. *Journal of Non-Crystalline Solids* 271(3):236-248.
48. Schmitt EA, Law D, Zhang GGZ 1999. Nucleation and crystallization kinetics of hydrated amorphous lactose above the glass transition temperature. *Journal of Pharmaceutical Sciences* 88(3):291-296.
49. Aso Y, Yoshioka S, Zhang J, Zografi G 2002. Effect of water on the molecular mobility of sucrose and poly(vinylpyrrolidone) in a colyophilized formulation as measured by C-13-NMR relaxation time. *Chemical and Pharmaceutical Bulletin* 50(6):822-826.
50. Buckton G 2000. Isothermal microcalorimetry water sorption experiments: calibration issues. *Thermochimica Acta* 347(1-2):63-71.
51. Lehto VP, Laine E 2000. Simultaneous determination of the heat and the quantity of vapor sorption using a novel microcalorimetric method. *Pharmaceutical Research* 17(6):701-706.
52. Sebhatu T, Angberg M, Ahlneck C 1994. Assessment of the Degree of Disorder in Crystalline Solids by Isothermal Microcalorimetry. *International Journal of Pharmaceutics* 104(2):135-144.
53. Hancock BC, Zografi G 1994. The Relationship between the Glass-Transition Temperature and the Water-Content of Amorphous Pharmaceutical Solids. *Pharmaceutical Research* 11(4):471-477.
54. Crowley KJ, Zografi G 2002. Water vapor absorption into amorphous hydrophobic drug/poly(vinylpyrrolidone) dispersions. *Journal of Pharmaceutical Sciences* 91(10):2150-2165.
55. Buckton G, Darcy P 1996. Water mobility in amorphous lactose below and close to the glass transition temperature. *International Journal of Pharmaceutics* 136(1-2):141-146.
56. Stubberud L, Forbes RT 1998. The use of gravimetry for the study of the effect of additives on the moisture-induced recrystallisation of amorphous lactose. *International Journal of Pharmaceutics* 163(1-2):145-156.
57. Berggren J. 2003. *Engineering of Pharmaceutical Particles - Modulation of Particle Structural Properties, Solid-State Stability and Tableting Behaviour by the Drying Process*. Department of Pharmacy, ed., Uppsala: Uppsala University. p 58.
58. Newell HE, Buckton G, Butler DA, Thielmann F, Williams DR 2001. The use of inverse phase gas chromatography to study the change of surface energy of amorphous lactose as a function of relative humidity and the processes of collapse and crystallisation. *International Journal of Pharmaceutics* 217(1-2):45-56.
59. Darcy P, Buckton G 1997. The influence of heating/drying on the crystallisation of amorphous lactose after structural collapse. *International Journal of Pharmaceutics* 158(2):157-164.
60. Mehnert W, Mader K 2001. Solid lipid nanoparticles - Production, characterization and applications. *Advanced Drug Delivery Reviews* 47(2-3):165.
61. Wissing SA, Kayser O, Muller RH 2004. Solid lipid nanoparticles for parenteral drug delivery. *Advanced Drug Delivery Reviews* 56(9):1257-1272.
62. Rao GCS, Kumar MS, Mathivanan N, Rao MEB 2004. Nanosuspensions as the most promising approach in nanoparticulate drug delivery systems. *Pharmazie* 59(1):5-9.
63. Corveleyn S, Remon JP 1998. Formulation of a lyophilized dry emulsion tablet for the delivery of poorly soluble drugs. *International Journal of Pharmaceutics* 166(1):65-74.

64. Pedersen GP, Faldt P, Bergenstahl B, Kristensen HG 1998. Solid state characterisation of a dry emulsion: a potential drug delivery system. *International Journal of Pharmaceutics* 171(2):257-270.
65. Yang SC, Zhu JB, Lu Y, Liang BW, Yang CZ 1999. Body distribution of camptothecin solid lipid nanoparticles after oral administration. *Pharmaceutical Research* 16(5):751-757.
66. Zimmermann E, Muller RH 2001. Electrolyte- and pH-stabilities of aqueous solid lipid nanoparticle (SLN (TM)) dispersions in artificial gastrointestinal media. *European Journal of Pharmaceutics and Biopharmaceutics* 52(2):203-210.
67. Hu FQ, Hong Y, Yuan H 2004. Preparation and characterization of solid lipid nanoparticles containing peptide. *International Journal of Pharmaceutics* 273(1-2):29-35.
68. Ugazio E, Cavalli R, Gasco MR 2002. Incorporation of cyclosporin A in solid lipid nanoparticles (SLN). *International Journal of Pharmaceutics* 241(2):341-344.
69. Freitas C, Muller RH 1998. Spray-drying of solid lipid nanoparticles (SLN (TM)). *Eur J Pharmaceutics and Biopharmaceutics* 46(2):145.
70. Muller RH, Radtke M, Wissing SA 2002. Nanostructured lipid matrices for improved microencapsulation of drugs. *International Journal of Pharmaceutics* 242(1-2):121-128.
71. Jenning V, Thunemann AF, Gohla SH 2000. Characterisation of a novel solid lipid nanoparticle carrier system based on binary mixtures of liquid and solid lipids. *International Journal of Pharmaceutics* 199(2):167-177.
72. Lindblom G, Rilfors L 1989. Cubic Phases and Isotropic Structures Formed by Membrane-Lipids - Possible Biological Relevance. *Biochimica Et Biophysica Acta* 988(2):221-256.
73. Landau EM, Rosenbusch JP 1996. Lipidic cubic phases: A novel concept for the crystallization of membrane proteins. *Proc Natl Acad Sci U S A* 93(25):14532-14535.
74. Ericsson B, Eriksson PO, Lofroth JE, Engstrom S 1991. Cubic Phases as Delivery Systems for Peptide Drugs. *Acs Symposium Series* 469:251-265.
75. Boyd BJ 2003. Characterisation of drug release from cubosomes using the pressure ultrafiltration method. *International Journal of Pharmaceutics* 260(2):239-247.
76. Larsson K 1999. Colloidal dispersions of ordered lipid-water phases. *Journal of Dispersion Science and Technology* 20(1-2):27-34.
77. Spicer PT, Small WB, Lynch ML, Burns JL 2002. Dry powder precursors of cubic liquid crystalline nanoparticles (cubosomes). *Journal of Nanoparticle Research* 4(4):297-311.
78. Craig DQM 1995. A Review of Thermal Methods Used for the Analysis of the Crystal Form, Solution Thermodynamics and Glass-Transition Behavior of Polyethylene Glycols. *Thermochim Acta* 248:189-203.
79. Craig DQM 2002. The mechanisms of drug release from solid dispersions in water-soluble polymers. *International Journal of Pharmaceutics* 231(2):131-144.
80. Wulff M, Alden M 1995. Phase-Equilibria in Drug-Polymer-Surfactant Systems. *Thermochim Acta* 256(1):151-165.
81. Wulff M, Alden M, Craig DQM 1996. An investigation into the critical surfactant concentration for solid solubility of hydrophobic drug in different polyethylene glycols. *International Journal of Pharmaceutics* 142(2):189-198.

82. Sjobkvist E, Nystrom C, Alden M 1991. Physicochemical Aspects of Drug Release .13. The Effect of Sodium Dodecyl-Sulfate Additions on the Structure and Dissolution of a Drug in Solid Dispersions. *International Journal of Pharmaceutics* 69(1):53-62.
83. Alden M, Tegenfeldt J, Sjobkvist E 1992. Structure of Solid Dispersions in the System Polyethylene Glycol-Griseofulvin with Additions of Sodium Dodecyl-Sulfate. *International Journal of Pharmaceutics* 83(1-3):47-52.
84. Wulff M, Alden M, Tegenfeldt J 2002. Solid-state NMR investigation of indomethacin-cyclodextrin complexes in PEG 6000 carrier. *Bioconjugate Chemistry* 13(2):240-248.
85. Pielichowski K, Flejtuch K 2003. Differential scanning calorimetry study of blends of poly(ethylene glycol) with selected fatty acids. *Macromolecular Material Engineering* 288(3):259-264.
86. Yeh F, Hsiao BS, Chu B, Sauer BB, Flexman EA 1999. Effect of miscible polymer diluents on the development of lamellar morphology in poly(oxymethylene) blends. *Journal of Polymer Science Pt B-Polymer Physics* 37(21):3115-3122.
87. Shieh YT, Lin YG, Chen HL 2002. Effect of supercritical CO<sub>2</sub> on phase structure of PEO/PVAc blends evaluated from SAXS absolute intensity measurement. *Polymer* 43(13):3691-3698.
88. Huang CI, Chen JR 2001. Crystallization and chain conformation of semicrystalline and amorphous polymer blends studied by wide-angle and small-angle scattering. *Journal of Polymer Science Pt B-Polymer Physics* 39(21):2705-2715.
89. Dreezen G, Ivanov DA, Nysten B, Groeninckx G 2000. Nano-structured polymer blends: phase structure, crystallisation behaviour and semi-crystalline morphology of phase separated binary blends of poly(ethylene oxide) and poly(ether sulphone). *Polymer* 41(4):1395-1407.
90. Danesh A, Chen X, Davies MC, Roberts CJ, Sanders GHW, Tendler SJB, Williams PM, Wilkins MJ 2000. Polymorphic discrimination using atomic force microscopy: Distinguishing between two polymorphs of the drug cimetidine. *Langmuir* 16(2):866-870.
91. Dey FK, Cleaver JAS, Zhdan PA 2000. Atomic force microscopy study of adsorbed moisture on lactose particles. *Advances in Powder Technology* 11(4):401-413.
92. Sindel U, Zimmermann I 2001. Measurement of interaction forces between individual powder particles using an atomic force microscope. *Powder Technology* 117(3):247-254.
93. Louey MD, Mulvaney P, Stewart PJ 2001. Characterisation of adhesional properties of lactose carriers using atomic force microscopy. *Journal of Pharmaceutical and Biomedical Analysis* 25(3-4):559-567.
94. Trojak A, Kocevar K, Musevic I, Srcic S 2001. Investigation of the felodipine glassy state by atomic force microscopy. *International Journal of Pharmaceutics* 218(1-2):145-151.
95. Beekmans LGM, van der Meer DW, Vancso GJ 2002. Crystal melting and its kinetics on poly(ethylene oxide) by in situ atomic force microscopy. *Polymer* 43(6):1887-1895.
96. Price R, Young PM 2004. Visualization of the crystallization of lactose from the amorphous state. *Journal of Pharmaceutical Sciences* 93(1):155-164.
97. Bond L, Allen S, Davies MC, Roberts CJ, Shivji AP, Tendler SJB, Williams PM, Zhang JX 2002. Differential scanning calorimetry and scanning thermal microscopy

- analysis of pharmaceutical materials. *International Journal of Pharmaceutics* 243(1-2):71-82.
98. Craig DQM, Kett VL, Andrews CS, Royall PG 2002. Pharmaceutical applications of micro-thermal analysis. *Journal of Pharmaceutical Sciences* 91(5):1201-1213.
  99. Royall PG, Kett VL, Andrews CS, Craig DQM 2001. Identification of crystalline and amorphous regions in low molecular weight materials using microthermal analysis. *Journal of Physical Chemistry B* 105(29):7021-7026.
  100. Aldén M, Lyden M, Tegenfeldt J 1994. Effect of Counterions on the Interactions in Solid Dispersions between Polyethylene-Glycol, Griseofulvin and Alkali Dodecyl Sulfates. *International Journal of Pharmaceutics* 110(3):267-276.

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