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**Understanding solid-state transformations during
dehydration: new insights using vibrational spectroscopy and
multivariate modelling**

by

Karin Kogermann

ACADEMIC DISSERTATION

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ABSTRACT

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Many active pharmaceutical ingredients (APIs) have both anhydrate and hydrate forms. Due to the different physicochemical properties of solid forms, the changes in solid-state may result in therapeutic, pharmaceutical, legal and commercial problems. In order to obtain good solid dosage form quality and performance, there is a constant need to understand and control these phase transitions during manufacturing and storage. Thus it is important to detect and also quantify the possible transitions between the different forms. In recent years, vibrational spectroscopy has become an increasingly popular tool to characterise the solid-state forms and their phase transitions. It offers several advantages over other characterisation techniques including an ability to obtain molecular level information, minimal sample preparation, and the possibility of monitoring changes non-destructively in-line.

Dehydration is the phase transition of hydrates which is frequently encountered during the dosage form production and storage. The aim of the present thesis was to investigate the dehydration behaviour of diverse pharmaceutical hydrates by near-infrared (NIR), Raman and terahertz pulsed spectroscopic (TPS) monitoring together with multivariate data analysis. The goal was to reveal new perspectives for investigation of the dehydration at the molecular level. Solid-state transformations were monitored during dehydration of diverse hydrates on hot-stage. The results obtained from qualitative experiments were used to develop a method and perform the quantification of the solid-state forms during processing-induced dehydration in a fluidised bed dryer. Both *in situ* and in-line process monitoring and quantification was performed.

This thesis demonstrated the utility of vibrational spectroscopy techniques and multivariate modelling to monitor and investigate dehydration behaviour *in situ* and during fluidised bed drying. All three spectroscopic methods proved complementary in the study of dehydration. NIR spectroscopy models could quantify the solid-state forms in the binary system, but were unable to quantify all the forms in the quaternary system. Raman spectroscopy models on the other hand could quantify all four solid-state forms that appeared upon isothermal dehydration. The speed of spectroscopic methods makes them applicable for monitoring dehydration and the quantification of multiple forms was performed during phase transition. Thus the solid-state structure information at the molecular level was directly obtained. TPS detected the intermolecular phonon modes and Raman spectroscopy detected mostly the changes in intramolecular vibrations. Both techniques revealed information about the crystal structure changes. NIR spectroscopy, on the other hand was more sensitive to water content and hydrogen bonding environment of water molecules. This study provides a basis for real time process monitoring using vibrational spectroscopy during pharmaceutical manufacturing.

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Helsinki, 2008

A handwritten signature in black ink that reads "Karin Kogermann". The script is cursive and elegant, with the first letters of "Karin" and "Kogermann" being significantly larger and more stylized than the rest of the letters.

Karin Kogermann

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List of abbreviations and mathematical symbols

Abbreviations

AFM	Atomic force microscopy
API	Active pharmaceutical ingredient
ASTM	The American Society of Testing and Materials
ATR	Attenuated Total Reflection
CBZ	Carbamazepine
CSD	Cambridge Structural Database
DRIFT	Diffuse reflectance infrared Fourier transform
DSC	Differential scanning calorimetry
EMEA	The European Medicines Agency
FDA	The Food and Drug Administration (United States)
ICH	International Conference on Harmonisation
IR	Infrared
MIR	Mid-infrared
NMR	Nuclear magnetic resonance
NIR	Near-infrared
PAT	Process analytical technology
PCA	Principal component analysis
PE	Polyethylene
Ph.Eur.	European Pharmacopoeia
PITs	Processing-induced transformations
PLS	Partial least squares
PLS-DA	Partial least squares discriminant analysis
PRX	Piroxicam
PTFE	Poly(tetrafluoroethylene)
RH	Relative humidity
RMSEC	Root mean square error of calibration
RMSEP	Root mean square error of prediction
RSD	Relative standard deviation
SEM	Scanning electron microscopy
SNV	Standard normal variate
TGA	Thermogravimetric analysis
TP	Theophylline
TPS	Terahertz pulsed spectroscopy
VT-XRPD	Variable temperature X-ray powder diffraction
XRPD	X-ray powder diffraction

Symbols

Q^2	Quantitative measure of the goodness of prediction, test-set validation coefficient
p	Loadings in PCA
PC	Principal component
R^2	Quantitative measure of the goodness of fit, regression coefficient
t	Score values in PCA and PLS
w	Weights in PLS

List of original publications

This thesis is based on the following original papers, which are referred to in the text by the Roman numerals **I - V**.

- I. Kogermann, K.,** Aaltonen, J., Strachan, C. J., Pöllänen, K., Veski, P., Heinämäki, J., Yliruusi, J., Rantanen, J. 2007. Qualitative *in situ* analysis of multiple solid-state forms using spectroscopy and partial least squares discriminant modeling. *Journal of Pharmaceutical Sciences*, **96**, (7), 1802 - 1820. DOI:10.1002/jps.20840

- II.** Zeitler, J. A., **Kogermann, K.,** Rantanen, J., Rades, T., Taday, P. F., Pepper, M., Aaltonen, J., Strachan, C. J. 2007. Drug hydrate systems and dehydration processes studied by terahertz pulsed spectroscopy. *International Journal of Pharmaceutics*, **334** (1 - 2), 78 - 84. DOI:10.1016/j.ijpharm.2006.10.027

- III. Kogermann, K.,** Zeitler, J. A., Rantanen, J., Rades, T., Taday, P. F., Pepper, M., Heinämäki, J., Strachan, C. J. 2007. Investigating dehydration from compacts using terahertz pulsed, Raman and near-infrared spectroscopy. *Applied Spectroscopy*, **61** (12), 1265 - 1274. DOI: 10.1366/000370207783292136

- IV.** Aaltonen, J., **Kogermann, K.,** Strachan, C. J., Rantanen, J. 2007. In-line monitoring of solid state transitions during fluidisation. *Chemical Engineering Science*, **62**, 408 - 415. ISSN:00092509

- V. Kogermann, K.,** Aaltonen, J., Strachan, C. J., Pöllänen, K., Heinämäki, J., Yliruusi, J., Rantanen, J. 2008. Establishing quantitative in-line analysis of multiple solid-state transformations during dehydration. (accepted in the *Journal of Pharmaceutical Sciences*)

1 INTRODUCTION

Most pharmaceuticals are marketed as solid dosage forms. During manufacturing the active pharmaceutical ingredients (APIs) are exposed to variable environmental conditions and encounter multiple stresses throughout the production cycle. Therefore, there is a significant possibility that phase transformations may occur. Similarly, solid-state changes may occur during storage. Solid-state conversions may involve a polymorphic transition from one polymorph to another, solvate formation or desolvation of a solvate, or interconversion to an amorphous form (Morris, Griesser et al. 2001). The consequences of these solid state changes may affect the final performance of the API. As a result the product quality, stability and also its therapeutic effects may be different to what was expected (e.g., reduced therapeutic effects, toxicity problems, Bernstein 2002). It is essential to discover possible phase changes during pharmaceutical development processes, and if possible, take precautions to control them, in part due to legal and commercial implications (Brittain and Fiese 1999; Bernstein 2002).

Crystalline hydrates are frequently encountered because water is prevalent in manufacturing of dosage forms. Many APIs when exposed to water may form hydrates, and on the contrary, hydrates may lose their water under high temperature or low humidity. Therefore the possibility of hydrate formation and dehydration during manufacturing or storage requires verification. For example, dehydration may occur during several unit operations, such as drying, milling, tableting, and as a result, the metastable anhydrous or amorphous forms may be obtained (*Fig. 1*). If these processes are not sufficiently controlled, partial conversion may occur and this may consequently impair the solid dosage form performance (Morris 1999).

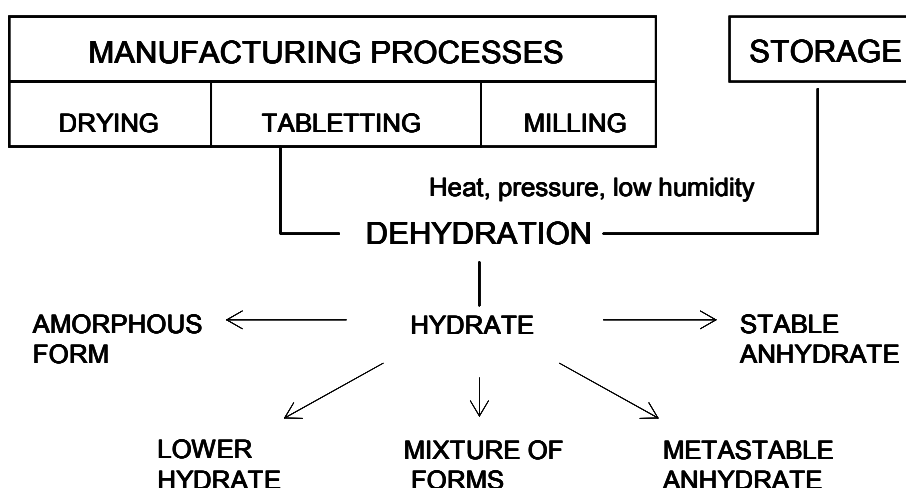


Figure 1. Conditions inducing dehydration and possible solid-state transformations.

INTRODUCTION

Several guidelines and strategies have been developed which list the analytical methods and tests that can be used to help the characterisation of hydrate-anhydrate systems (Byrn, Pfeiffer et al. 1995). Proposed techniques allow the full characterisation of hydrates *in situ* after preliminary crystallisation which is needed for scientific and regulatory purposes. However, methods are also needed for further steps in drug development and manufacturing providing information about the behaviour of APIs under processing conditions. According to the Food and Drug Administration guidelines, the "*quality cannot be tested into products; it should be built-in or should be by design*" (FDA 2004). Final product testing and a process validation alone, do not guarantee a sufficient quality, efficacy and control. The in-line, on-line and at-line monitoring with process analytical technology (PAT) tools allow deeper insight into process understanding and can reveal the potential phase transformations during manufacturing. Thus, the stability of final product upon storage can be assured.

Recently vibrational spectroscopy techniques have gained an important place as PAT tools for in-line process monitoring. Spectroscopic techniques are fast, non-destructive and non-invasive, and probe the changes at the molecular level. When spectroscopy is combined with appropriate data analysis the process can be both qualitatively and quantitatively assessed. Thus complete information about the solid-state changes is obtained and the behaviour of an API in real process environments can be determined. Multivariate data analysis can reveal information about multiple variables that are involved in process, their relationships and correlations, and provide information on different processing steps and the state of the process throughout manufacturing (Eriksson, Johansson et al. 2001).

The prediction and control of possible processing-induced phase transformations (PITs) may be complicated. To adequately control the process, changes in hydration state need to be examined and mechanisms of conversions understood. Therefore, there is a constant need to monitor the dehydration behaviour of an API and assess the effect of dehydration conditions. In addition to revealing the dehydration behaviour of powder samples, APIs are often compacted during several steps of processing. The dehydration mechanisms from compacted samples (such as tablets) are governed by the diffusion of crystal water through the physical matrix. Therefore, the dehydration under those conditions should also be investigated. This provides some insight into dehydration during processing and storage of compacts.

Drying is one of the most critical manufacturing processes during which dehydration of hydrates is frequently encountered. Thus the drying parameters should be properly selected to obtain the appropriate solid-state form. The knowledge obtained from *in situ* experiments allows

INTRODUCTION

prediction of the behaviour of APIs in real process environments. In-line process monitoring with vibrational spectroscopy and modelling allows the mechanisms and kinetics of possible solid-state transformations to be investigated. Consequently, real time process control can be achieved.

In this thesis, the dehydration behaviour of pharmaceutical hydrates was assessed *in situ* and in a real process environment during fluidised bed drying. Vibrational spectroscopy was used to monitor the solid-state transformations and multivariate data analysis was performed to interpret the spectral information. In addition, the *in situ* dehydration behaviour was investigated from compacts. The overall goal was to develop a method for monitoring the dehydration behaviour and predict and quantify the multiple solid-state transformations in-line during drying.

2 THEORETICAL BACKGROUND AND REVIEW OF THE LITERATURE

This section provides an overview of the importance of studying solid-state transformations of hydrates. The theoretical aspects of the dehydration and the analytical methods are introduced and discussed.

2.1 *Introduction to solid state properties of pharmaceutical materials*

The basic characteristics describing the solid state are a fixed shape, strong intermolecular forces between the atoms and molecules, and less translational motion compared to gaseous or liquid phases. In the pharmaceutical manufacturing of solid dosage forms, important properties also include surface energy, hardness, compaction, elastic properties and porosity (Mansoor and Sandmann 2003). The ease of product handling and high stability favour the use and manufacturing of solid dosage forms.

Pharmaceutical materials may exist in different solid states. They can be either in a crystalline form, where the atoms and molecules are arranged in a repetitive manner in a crystal lattice and there is a long range order of molecules, or in an amorphous form, where there is a lack of long-range order, and only short range order derived from short-range intermolecular forces has been observed (Grant 1999). Moreover, several pharmaceutical solids may exist in more than one crystalline form, thus exhibiting polymorphism (Greek: polus = many; morph = form, Grant 1999; Bernstein 2002). Polymorphism is defined as the ability of a compound with the same chemical identity to crystallize in different crystal forms where the molecules inside the crystal lattice have different arrangements and/ or conformations (Grant 1999). For flexible molecules the occurrence of multiple molecular conformations in different crystal structures is referred to as conformational polymorphism (Bernstein 2002).

In addition to polymorphs and the amorphous solid state, other examples of possible solid states are solvates and hydrates, which have recently been termed solvatomorphs (Brittain 2007). Solvates and hydrates are crystalline forms that include solvent molecules (or water molecules in case of hydrates) in their respective crystal lattice (Morris 1999). Hydrates are frequently encountered solvates in pharmacy, because water is applied for many processing steps. Until recently, the solvatomorphs were referred to in the literature as pseudopolymorphs, but this term should no longer be used because of misunderstandings and confusions (Seddon 2004; Bernstein 2005).

2.2 *Physicochemical properties and the pharmaceutical implications of different solid-state forms*

Physicochemical properties (melting point, density, solubility, dissolution rate) and pharmaceutical properties (particle habit, powder flow, hardness, and compressibility) can considerably vary between solid-state forms (Grant 1999). This, may lead to differences in bioavailability and stability, and consequently affect the pharmaceutical performance of the solid dosage form (Halebian and McCrone 1969). A well-known example is the case of ritonavir, a protease inhibitor for human immunodeficiency virus, where the formation of a more stable form resulted in precipitation of the form from a semisolid formulation and dissolution test failures (Chemburkar, Bauer et al. 2000). Therefore, one important factor affecting product quality is the selection of the solid phase. Proper characterisation of the initial solid-state form and its behaviour under different environmental conditions (temperature, pressure, and humidity), allows final product to be formulated and manufactured with the desired properties (Zhang, Law et al. 2004).

Polymorphs exhibit differences in thermodynamic stability, and they can be divided into two main classes. When one polymorph is stable at all temperatures and pressures below the melting point, and all other polymorphs are unstable, the polymorphic system is referred to as monotropic (*Fig. 2*).

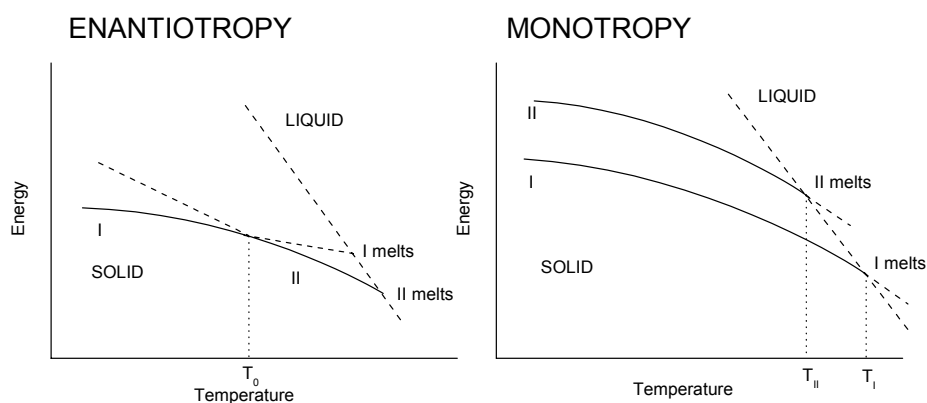


Figure 2. Gibbs free energy in relation to temperature for enantiotropic and monotropic systems (modified from Giron et al., 1995).

In this case the free energy curves do not intersect below the melting point, and no reversible transition temperature below melting is thus observed. However, in an enantiotropic system, one polymorph is stable up to a transition temperature, above which another polymorph is stable (Burger and Ramberger 1979a). The free energy curves cross below the melting point, and a solid-state transformation occurs from one polymorphic form to another. The most stable polymorph has the lowest Gibbs free energy, vapour pressure, thermodynamic activity, fugacity, solubility and dissolution rate, and rate of reaction. It is known, that higher thermodynamic activity increases the apparent solubility and dissolution rate in a particular solvent. Thus, higher chemical and thermodynamic reactivity results in lower stability.

It is important to distinguish hydrates from real polymorphs, as hydrates are molecular adducts consisting of water molecules in their crystal lattice. Polymorphs and hydrates differ in their free energy relationship with the environmental conditions. In the case of polymorphs, where we are dealing with one component system, the free energy is defined by temperature and pressure. However, with hydrates, a two component system is involved and the free energy is also affected by water activity (Morris 1999).

2.3 *Pharmaceutical hydrates*

Approximately one third of APIs are capable of forming a hydrate form (Threlfall 1995). Hydrates seem to be more common among larger molecules (Bernstein 2002). The presence of water molecules changes the crystal structure of an initial anhydrate or lower hydrate, such as the dimension, shape, symmetry and capacity of a unit cell. As a consequence, the intermolecular interactions and bonding within the solid cause changes in the internal energy and enthalpy. Changes in entropy together with those in enthalpy result in free energy changes. This, on the other hand, leads to changes in thermodynamic activity, solubility and chemical stability as discussed in the previous paragraph.

Commonly, anhydrous forms have higher aqueous solubility and dissolution rates than hydrates, which may lead to increased bioavailability, when dissolution is the rate limiting step for drug absorption. The free energy change for the anhydrate form is higher, because hydrates have already interacted with water. Lowered bioavailability has been reported with hydrate forms of several APIs (Shefter and Highuchi 1963; Halebian, Koda et al. 1971; Rodriguez-Hornedo,

Lechuga-Ballestros et al. 1992). However, hydrates are typically the most stable forms in aqueous solutions and under high humidity conditions (Byrn, Pfeiffer et al. 1999).

Several APIs can exhibit multiple hydrated states. For example, nedocromil sodium, used in the treatment of reversible obstructive airway diseases, which can exist in different hydrated states termed heptahemihydrate, trihydrate, and monohydrate, and as an amorphous form consisting of variable amounts of water (Khankari, Chen et al. 1998). The most hydrated form is the stable form at the lowest temperature and ambient pressure (Giron 1995). In addition, polymorphism of hydrates has been reported (Haleblian, Koda et al. 1971; Pienaar, Caira et al. 1993). In this case the hydrate forms have the same chemical composition but different molecular rearrangements. It is, however, important that the two forms of hydrates have the same stoichiometry to be called polymorphs (Khankari and Grant 1995).

Solubility and stability differences between anhydrous and hydrate forms determine the choice of the preferred form for production. The general rule is that when a hydrate is only formed in aqueous solution, and no transformation from anhydrate to hydrate occurs at a high relative humidity ($RH \geq 80\%$), the anhydrous form is preferred (Giron 1995). This is not, however, the case with all pharmaceutical compounds. A number of excipients (magnesium stearate, lactose, glucose) and other APIs, such as antibiotics ampicillin (Shefter, Fung et al. 1973) and erythromycin (Laine, Kahela et al. 1987), and vitamin B₁₂ (cyanocobalamine, Haleblian 1975) are marketed in a hydrate form, which is the more stable form at ambient temperature and humidity. For example, anhydrous cefixime, cephalosporin antibiotic, is physically unstable, and under humid conditions it reversibly transforms to a hydrate form. Thus cefixime trihydrate is selected for production (Kitamura, Koda et al. 1990). If the hydrate is formed already under low humidity ($RH \leq 65\%$), the choice of the preferred form is more difficult (Giron 1995). For example, in case of carbamazepine the formation of hydrate form during storage under humid conditions has been reported (Kaneniwa, Yamaguchi et al. 1984; Meyer, Straughn et al. 1992; Wang, Shiu et al. 1993).

Classification of hydrates facilitates a great deal in revealing the characteristics and the subsequent behaviour of new hydrates. Hydrates have been classified according to the energetic state and to the structure (Morris 1999). The structural classification allows predicting the thermodynamic behaviour of a hydrate based on the knowledge about the crystal structure, or conversely the thermodynamic analysis facilitates some insight into the structure of hydrate. There are two different approaches applied in the literature, where structural classification has been proposed.

One structural classification divides hydrates into three main categories (Morris 1999). The first category involves isolated-site hydrates, where water molecules are separated from direct contact to one another by intervening drug molecules, one example is nitrofurantoin monohydrate (antibacterial drug). The second type comprises channel hydrates, where water molecules are connected with each other by hydrogen bonding, and form a tunnel-like structure through the crystal, with an example being carbamazepine dihydrate (*Fig. 3*). This class can be subclassified into three sub-classes, expanded channel or non-stoichiometric hydrates, planar channel hydrates and dehydrated hydrates or isomorphous hydrates. Expanded channel hydrates are able to take additional amounts of water into their structure under high humidity by expanding the crystal structure, e.g. cromolyn sodium (antiallergic drug). Planar hydrates comprise water which is in a two-dimensional order, e.g. sodium ibuprofen (anti-inflammatory drug). Dehydrated hydrates obtain similar crystal structure with the hydrate form upon water removal only with reduced packing efficiency and therefore with lowered stability (lower density), for example isomorphous dehydrate of erythromycin (Stephenson, Stowell et al. 1997). The third class of hydrates is ion-associated hydrates. This category involves metal-ion coordinated water in the structure, for example calteridol calcium tetrahydrate (chelating excipient in parenteral formulations, Morris 1999).

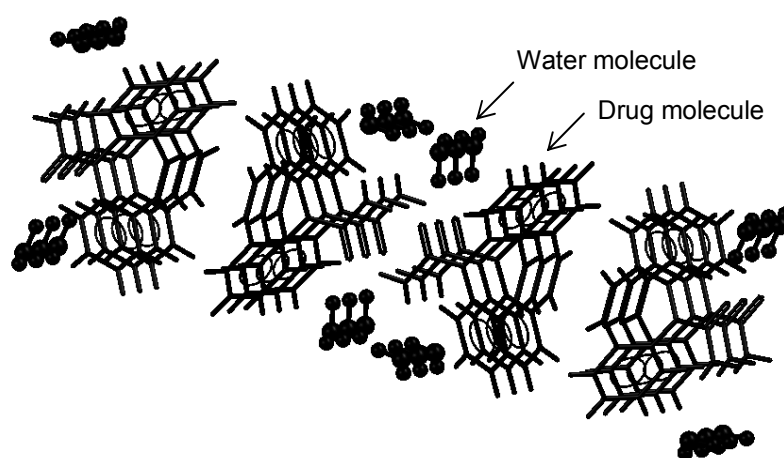


Figure 3. Example of channel hydrate carbamazepine dihydrate, obtained from CSD.

An alternative method of classification according to the structure, divides hydrates as stoichiometric hydrates, where water content is strictly defined, and as non-stoichiometric hydrates, where the water content varies (Authelin 2005; Griesser 2006). A detailed review of the thermodynamic behaviour of non-stoichiometric hydrates has been published by Authelin et al. (Authelin 2005) and a discussion about the properties of stoichiometric hydrates is reported by Khankari and Grant (Khankari and Grant 1995).

2.3.1 Phase transitions of hydrates

Solid-state transformations between hydration states may occur for many pharmaceutical compounds and cause problems in pharmaceutical manufacture, because the final product quality may differ from the initially chosen form for production. Hydrates may be formed when an API comes in contact with water during manufacturing (for example during wet granulation, pelletisation, crystallisation) or during storage (under high humidity). For example, during wet granulation carbamazepine anhydrous forms transform to carbamazepine dihydrate (Otsuka, Hasegawa et al. 1999). In addition, under high humidity conditions, the crystallisation of theophylline monohydrate in tablets (Herman, Visavarungroj et al. 1989; Ando, Ishii et al. 1992; Adeyeye, Rowley et al. 1995) and pellets upon storage (Herman, Remon et al. 1988; Herman, Visavarungroj et al. 1989) has been reported. Other examples involve nitrofurantoin and caffeine (stimulant), where the phase transformation to a hydrate form has been observed in high humidity (Shefter and Highuchi 1963; Otsuka, Teraoka et al. 1991; Ando, Ishii et al. 1992).

Another possible phase transition of hydrates that may occur during both the manufacturing and storage is dehydration. This is a phase transition from crystalline hydrate to anhydrate (crystalline or amorphous anhydrate), or from higher hydrate to lower hydrate (Morris 1999). Upon dehydration water molecules leave the crystal structure due to the effect of water vapour pressure or temperature, or due to the effect of mechanical stress brought into the system. Caffeine 4/5 hydrate is known to lose its water already under ambient conditions (Byrn, Pfeiffer et al. 1999) and cefixime trihydrate becomes unstable during storage below its critical relative humidity (Kitamura, Koda et al. 1990). Cromolyn sodium and cefazolin sodium are examples of non-stoichiometric channel hydrates. The hydration state of those hydrates is reversible and is the function of relative humidity (Cox, Woodard et al. 1971; Stephenson and Diserod 2000). Thus the stability and performance of those hydrates in formulation are highly affected by the storage conditions.

2.3.2 Dehydration mechanisms

The dehydration may comprise different solid-state transformations, and the corresponding product and its stability depends largely on the dehydration mechanisms and conditions (*Fig. 4*). The dehydration may result in formation of an amorphous form (Han, Gupte et al. 1998), a different crystal structure, or even a mixture of forms. This has been referred as hard or

destructive dehydration. The initial crystal structure might also be retained during the dehydration, and is known as smooth dehydration.

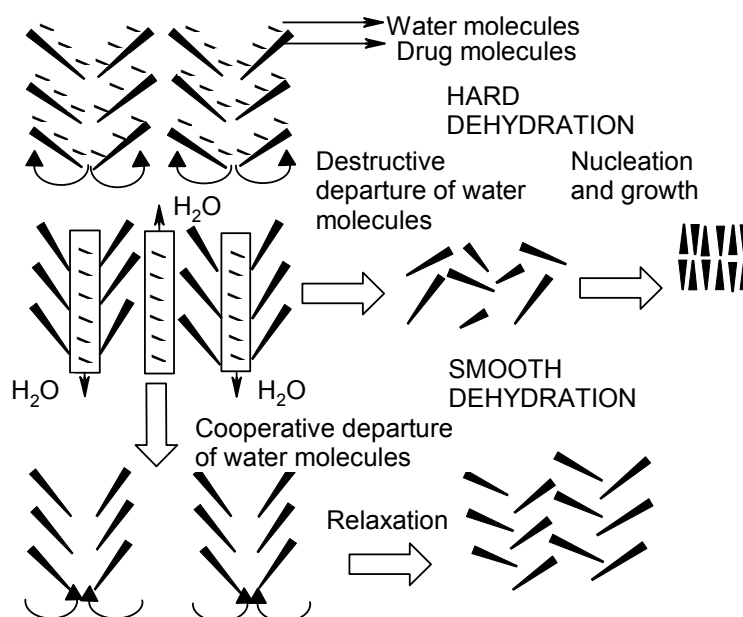


Figure 4. Different dehydration mechanisms showing the simplified explanation to the obtained solid-state forms (modified from Garnier et al., 2002).

For example, a two-step process has been reported for risedronate hemi-pentahydrate (treatment of osteoporosis) dehydration (Redman-Furey, Dicks et al. 2005; Lester, Lubey et al. 2006). In the first step a metastable monohydrate is formed with one molecule of water removed, and the second heating step results in anhydrous form (Lester, Lubey et al. 2006). An anti-inflammatory drug, fenopropfen sodium dihydrate is unusually stable for a salt hydrate, and only during heating it directly transforms to anhydrous form. The crystal structures include totally different arrangements and hydrogen bonding, therefore the dehydration occurs through structural rearrangements in the crystal lattice (Stephenson and Diseroad 2000).

To assess the dehydration, it is of relevance to understand its underlying mechanisms and obtain additional information from the kinetic analysis of the phase transitions. Petit (Petit and Coquerel 1996), Galwey (Galwey 2000) and Zhang (Zhang, Law et al. 2004) have developed models and classified the dehydration behaviours. These classifications include the accepted reaction models and also theoretical principles, which are necessary if we want to predict drug behaviour during pharmaceutical processes. It is a valuable tool in the understanding of the decomposition of solid-state pharmaceuticals and together with reliable experimental data enables us to explain the different behaviour of hydrates.

Dehydration mechanisms include solid-state, solution or sometimes also melting mediated transformations (Zhang, Law et al. 2004). For solid-state mechanism, the phase transition occurs from solid to solid without intermediate liquid or gaseous phases. This kind of mechanism is highly affected by the environmental conditions (humidity, pressure, temperature), and presence of crystal defects, particle size and distribution, and impurities. When the solution mechanism is involved, then some of the API will dissolve in a solvent (e.g., water), and the transformation occurs after the removal of the solvent. In this case, the transformation may occur from a metastable form to a stable or from a stable form to a metastable, thus only the part of the API that dissolves will transform. The final product may either be a single form or a mixture of different solid-state forms depending on the rate of the water removal, and the nucleation and growth of possible crystal forms. The melting mechanism is not often encountered with dehydration, however in this case, the API is heated above the melting temperature and then cooled back to ambient temperature, and the initial state cannot be restored. Upon dehydration, the most important factors affecting the final product are the relative rates of nucleation, crystal growth, cooling, and the presence of impurities and excipients (Zhang, Law et al. 2004).

As can be seen, the dehydration mechanisms and the final solid-state form depend on several factors, such as the environmental conditions (Griesser and Burger 1995; Han and Suryanarayanan 1997; Suihko, Ketolainen et al. 1997; Han, Gupte et al. 1998) as well as on crystal packing, hydrogen bonding, crystal habit, crystal defects (nucleation sites), and sample and particle sizes (Byrn, Pfeiffer et al. 1999).

2.3.3 Processing of hydrates and processing-induced dehydration

Processing-induced dehydration may occur during several unit operations such as drying, mixing, milling and tableting. Several reports can be found from the literature that discuss about the phase transitions of hydrates caused by manufacturing conditions. For instance, carbamazepine dihydrate is unstable during compression, thus this form should be avoided for direct compression (Lefebvre, Guyot-Hermann et al. 1986). Grinding-induced formation of amorphous ampicillin from trihydrate (Han, Gupte et al. 1998) and amorphous cephalexin from monohydrate (Otsuka and Kaneniwa 1984) have been reported.

One of the most challenging tasks for pharmaceutical industry is to control the solid-state of an API during the drying unit operation. Both the preferred solid-state form and particle size may

not be suitable after drying, if the process is not properly controlled. When hydrates are involved, overdrying can be critical when the hydrate form is selected for production, or underdrying when the anhydrate form is preferred. Moreover, when an API has multiple anhydrous forms, the control over the final solid-state form is even more complicated (Morris, Nail et al. 1998). For example, theophylline is reported to transform to a monohydrate during wet granulation (Herman, Remon et al. 1988; Räsänen, Rantanen et al. 2001) and consequently transforms either to a metastable anhydrate or stable anhydrate upon drying phase depending on the drying rate and humidity (Airaksinen, Karjalainen et al. 2004). A metastable form has a tendency to transform to a stable anhydrate upon storage causing difficulties for product quality and control (Phadnis and Suryanarayanan 1997). This is based on the general phase rule that the transformation occurs into the direction of the more stable form, which has the lowest free energy. Another example, thiamine hydrochloride (vitamin B₆) transforms to a monohydrate during spray granulation, and during the final drying loses its water, and a dehydrated anhydrate is obtained. When a dehydrated anhydrate is exposed to ambient conditions during tableting, again the monohydrate is formed. After storage for four months at room temperature the monohydrate converts to a hemihydrate. As a result, the tablet hardness and disintegration times are increased (Wöstheinrich and Schmidt 2001).

When, during processing, there are multiple sources of variability and these variables are not properly controlled, additional *in situ* and real time studies are needed to obtain sufficient process control (ICH 2000). Final product testing alone cannot provide enough information about the variations. Solid-state transformations should be monitored and controlled during processing. Only then can the product stability and performance be assured. Furthermore, the analysis methods used for monitoring should be properly validated (ICH 1995).

It is important to understand the dehydration behaviour of an API to get an insight into the dehydration mechanisms encountered during processing. The main goal of pharmaceutical industry is to produce a final product with defined physicochemical and pharmaceutical properties. This, however, requires sufficient process understanding and control (ICH 2000; ICH 2006a; ICH 2006b). If we are able to predict the behaviour, we are also able to monitor and control the process. Process analytical technology or so called PAT is a term describing the methods for increasing process understanding and are applied for process control purposes (FDA 2004). PAT methods involve the analytical techniques applied for at-line, on-line and in-line process monitoring, chemometrics for analysing the process data and also the overall concept of design of experiments (FDA 2004). By combining all these methods, sufficient process understanding and then control can be achieved.

2.4 *Analytical techniques used to characterise hydrates and their solid-state transitions*

Table 1 lists techniques that can be used to characterise and analyse the solid-state forms, and highlights their main advantages and disadvantages (Brittain 1999; Vippagunta, Brittain et al. 2001). Changes in crystal structure or chemical composition (e.g. hydrates) and the corresponding differences in physicochemical properties can be used for the detection of solid-state forms and their transitions. Specific strategies together with a list of analytical techniques (Byrn, Pfeiffer et al. 1995) and also molecular modelling methods (Giron, Mutz et al. 2004) have been proposed to aid in the characterisation of hydrates.

Table 1. Analytical techniques to investigate and characterise the solid-state forms (modified from Giron, Mutz et al. 2004)

	Method	Data measured	Main advantages	Main disadvantages
Molecular level properties	FT-IR, DRIFT, ATR	Intramolecular vibrations (dipole moment changes)	Chemical identification (good fingerprint) Molecular structure information Rapid measurements Information about solvent and solvates Quantitation possible	Sample preparation artefacts possible Small differences Interference from excipients and humidity Probes are difficult to use in MIR region
	Raman spectroscopy	Intramolecular vibrations (polarisability changes)	Chemical identification Molecular structure information No sample preparation, rapid measurements, probe possible Ability to penetrate through containers Water is Raman inactive Relatively insensitive to particle size Quantitation possible	Interference from excipients Local sample heating Fluorescence Consider sample volume
	NIR spectroscopy	Overtone and combinations of molecular vibrations (dipole moment changes)	Chemical /physical information No sample preparation, rapid measurements Ability to penetrate through containers Ability to show different states of water Quantitation possible	Affected by water and particle size, low intensity Subtle differences, broad bands and overlapping regions Poor fingerprint
	Solid-state NMR	Magnetic resonance	Chemical information /directly probes atom positions Phase characterisation, crystal structure determination Minimal sample preparation Insensitive to particle size Quantitation possible	Heating of the sample by sample spinning Experimental artefacts Large sample Slow

THEORETICAL BACKGROUND AND REVIEW OF THE LITERATURE

Table 1. continues.

Particle level properties	X-ray diffraction Single crystal X-ray diffraction, XRPD	Diffractogram from single crystal from powder sample	Crystal structure (“golden standard”), phase identification Crystallinity measurements Quantitation possible	Influenced by particle size and orientation (preferred orientation) Difficulty preparing single crystal Interference from crystalline excipients Probes not possible, slow
	TPS	Intermolecular vibrations and molecular flexing (dipole moment changes)	Crystal structure information Additional information from molecular rotations in gaseous phase No sample preparation (ATR measurements), rapid measurements Quantitation possible	Affected by water vapour Diffuse reflectance set-up not currently available Difficulties in interpretation (modes currently not well understood)
	Optical microscopy/ hot-stage microscopy/ SEM, AFM	Microscopy under the influence of light or electron radiation	Morphology (shape, size, colour) Surface examination (optical constants/ interfacial angles) Qualitative information on crystallinity Melting, transition and eutectic point determinations Study dehydration, crystallisation	Interference from excipients Small scanning area Limited quantitative information
	DSC	Heat flow versus temperature	Fast, very sensitive, automation Best thermodynamic information Quantitative information about relative stability and the energies involved with phase change Glass transition temperature determination	Quantitation limited due to kinetic effects Impurities modify melting points
	TGA	Change of mass versus temperature	Fast, very sensitive, automation Study solvates/hydrates, phase transitions Release and stability testing Quantitation	No information about the nature of transition No structural information Interference from water containing excipients
	Bulk level properties	Microcalorimetry	Heat flow versus time	Quantitation of amorphous phase Formation and loss of hydrates
Solution calorimetry		Heat flow during dissolution	Detection and quantitation of polymorphs and amorphous phase Sensitive to low energy differences	Interference from other crystalline forms, Interactions in a particle (crystalline core and amorphous on the surface), Solubility problems
Moisture sorption/desorption isotherms		Change of mass versus RH%	Hygroscopicity behaviour Hydrate formation and stability Crystallisation of amorphous phase Detects low levels of amorphous phase	Interference from amorphous excipients Hysteresis possible
Karl Fischer titrimetry		Amount of water%	Total water content in sample	No separation between absorbed and crystal water
Density (pycnometry, flotation, indirectly using unit cell constants)		Mass per unit volume	Relative stability of polymorphs “Burger rule: more unstable form at zero degrees Celsius should have lower density”	Exceptions to the rule possible
Solubility		Amount dissolved in different solvents and temperatures	Solubility versus temperature transition point Saturation solubility - analysis of insoluble Solvent-mediated transition - stable form	Characteristic data needed Influence impurities Influence of change during measurement

Deeper insight into the process can be achieved, when complementary techniques are used (Giron, Goldbronn et al. 2002) and when measurements are performed under different environmental conditions (humidity-controlled DSC-XRD, Han, Zhang et al. 2003; pressure DSC, Han and Suryanarayanan 1997; Han, Gupte et al. 1998). Han and Suryanarayanan have proposed a rapid method to assess the physical stability of hydrates by using a humidity controlling device during thermal measurements (Han and Suryanarayanan 1999). To date, a number of analytical techniques have been developed that allow simultaneous measurements (FT-IR combined with Raman and hot-stage microscopy, Raman-XRPD, FT-IR or TGA or Raman and hot-stage microscopy, X-ray DSC), and in addition reveal extra information about the gaseous phase (e.g. combined TG-IR, Rodriquez and Bugay 1997).

Mid-infrared (MIR), near-infrared (NIR) and Raman spectroscopy are well established methods described in pharmacopoeias (Ph.Eur. 2005a; Ph.Eur. 2005b; Ph.Eur. 2005c), terahertz pulsed spectroscopy (TPS) is a relatively new developed method for solid-state analysis (Taday and Newnham 2004). Since all these techniques are suitable for process monitoring and quantification their basic aspects are covered in more detail together with examples of their pharmaceutical applications.

2.4.1 Mid-infrared spectroscopy

MIR spectroscopy exploits the electromagnetic radiation approximately at $4000 - 400 \text{ cm}^{-1}$. MIR spectra are produced when molecules change their vibrational energy states between the ground state and the first excited state (*Fig. 5*). MIR radiation probes the intramolecular vibrations of the fundamental groups. In order for molecular vibration to be active in infrared and absorb the infrared radiation, the change in the dipole moment needs to occur (Colthup, Daly et al. 1990). Hence, the polar groups, such as C-F, Si-O, C=O and C-O, absorb the infrared energy strongly, and antisymmetric stretches correspond with high intensity in infrared spectrum.

MIR spectrometers operate with a polychromatic light source, and the measurements can be performed either in diffuse reflectance (DRIFTS) or transmission mode (Nujol mulls). In addition, attenuated total reflection (ATR) measurements can be performed. For MIR measurements, usually Fourier transform technology is applied, thus the beam attenuation and transmission problems can be circumvented. Fourier transformation converts the interferogram into the spectrum (absorption in relation to wavenumber, Colthup, Daly et al. 1990; Bernstein 2002).

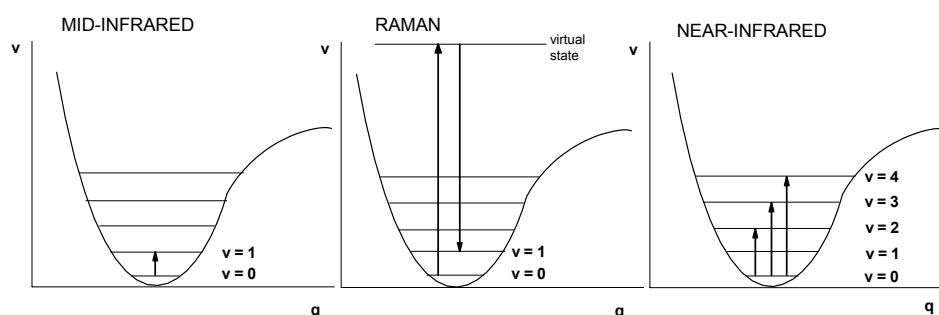


Figure 5. Vibrational energy level diagram for MIR, Raman, and NIR spectroscopy, adapted from (Siesler, Ozaki et al. 2002); v vibrational quantum number, q vibrational coordinate; $v = 0$ (ground state), and $v = 1, v = 2 \dots n$ (excited states).

Infrared spectroscopy allows identifying the solid-state forms and performing quantitative analysis. For quantitative analysis, MIR spectroscopy follows Beer's law (Equation 1):

$$\log \frac{I_0}{I} = A = a \times b \times c, \quad (1)$$

where **A** is absorbance, I_0 is the intensity of the incident light, I is the intensity after passing through the material, **a** is the molar absorptivity or absorption coefficient, **b** is the path length of the sample or the distance that the light travels through the material, **c** is the concentration of the absorbing materials

MIR spectroscopy reveals molecular properties rather than solid-state properties, however, the comprehensive band assignment allows distinction between solid-state forms and process monitoring at different conditions (e.g. heating/cooling) can be conducted. Typically sample preparation is required, unless an ATR accessory is applied. Pharmaceutical solids, including hydrates, can be characterised by MIR spectroscopy. The molecular vibrations and the corresponding hydrogen bonding are changed due to the presence of water molecules. Most of the differences are located at high energy region ($4000 - 2000 \text{ cm}^{-1}$) and more specifically within OH stretching region ($3600 - 3100 \text{ cm}^{-1}$). As an example, different hydrate forms of risnedronate have been differentiated using MIR spectroscopy (Redman-Furey, Dicks et al. 2005). However, subtle differences between the forms in MIR spectra may render the characterisation of the forms, as reported with hydrate forms of digoxin (Botha and Flanagan 1992). *In situ* ATR FT-IR technique has been successfully used for monitoring the crystallisation processes of APIs (Fevotte 2002; Liotta and Sabesan 2004). In addition DRIFTS-IR method combined with PLS analysis allowed quantification of the sulfathiazole polymorphic forms (Pöllänen, Häkkinen et al. 2005).

It is not possible to interface the light in MIR region with the probe hindering the in-line measurements (McCreery 2000), therefore the most common vibrational spectroscopy method - MIR spectroscopy was not used in this study.

2.4.2 Raman spectroscopy

Raman spectroscopy typically involves the region between 4000 and 200 cm^{-1} . With special set-ups, however, the far-IR region up to 10 cm^{-1} characterising the lower frequency lattice vibrations can be probed (Bolton and Prasad 1981). Raman spectra correspond to the polarisability changes in the sample induced by the incident monochromatic light. When the monochromatic light irradiates the sample, the potential energy of the molecules is increased to a higher energy state, and after this most molecules relax back to their initial energy level (*Fig. 5*). Therefore, most of the light is elastically scattered (Rayleigh scattering) in different directions. However, a small part (1 in 10^6 photons) of the radiation is inelastically scattered back and shifted in frequency. This inelastically scattered light corresponds to the Raman spectra of the material, with some of the radiation is shifted to a higher frequency (anti-Stokes bands) and some is shifted to a lower frequency (Stokes bands). Most of the Raman spectrometers used at room temperature detect the Stokes in Raman spectra (Colthup, Daly et al. 1990).

Raman spectroscopy can be considered as a complementary technique to infrared. While MIR is an absorbance method, Raman is a scattering technique. Conversely to infrared spectroscopy the radiation is more effectively scattered back from non-polar groups, and symmetric stretches of the molecules respond to higher intensity values in Raman spectrum (Colthup, Daly et al. 1990).

For the collection of Raman spectra, usually the Fourier transform Raman (FT-Raman) or dispersive Raman are applied (McCreery 2000). The techniques differ mainly in the laser source and the way Raman scattering is detected and analysed. In addition to backscattering mode Raman, recently the transmission Raman spectroscopy has been applied for pharmaceutical analysis of bulk solid dosage forms (Matousek and Parker 2006a; Matousek and Parker 2006b). This has an advantage that measurements are not limited by small sampling volume.

Raman spectroscopy can be used to determine the molecular structure, characterise between different solid-state forms, determine the hydration states and solid-state phase transitions and it can be applied for both qualitative and quantitative analysis of pharmaceuticals (Pelletier 2003). The solid-state forms of APIs have been identified in intact tablets using FT-Raman

spectroscopy, also allowing a clear separation between theophylline anhydrate and monohydrate forms (Taylor and Langkilde 2000). Quantification with binary mixtures of hydrate and anhydrate has been performed (Rantanen, Wikström et al. 2005a). For quantitative purposes, the Raman peak intensity (I_{Raman}) is directly proportional to the concentration (c) of the API (Pelletier 2003):

$$I_{Raman} \sim c$$

Rapid, non-destructive and non-invasive measurements allow the implementation of Raman spectroscopy in the pharmaceutical industry as a routine testing technique, which can be applied for real time process monitoring. For example, the applicability of FT-Raman for in-line monitoring of powder blending has been reported (Vergote, De Beer et al. 2004). Several authors have applied Raman spectroscopy for the analysis of solid-state characteristics and behaviour of drug hydrates. For example, the *in situ* monitoring of solvent-mediated transformation of progesterone has been reported using Raman spectroscopy (Wang, Wachter et al. 2000). The dehydration of caffeine hydrate was monitored using FT-Raman spectroscopy in an environmental chamber (De Matas, Edwards et al. 1998). In addition, the feasibility of Raman spectroscopy to monitor the solid-state changes during dehydration of carbamazepine dihydrate (McMahon, Timmins et al. 1996) and trehalose dihydrate *in situ* (Taylor, Williams et al. 1998) and during fluidised bed drying of risedronate sodium have been reported (Hausman, Cambron et al. 2005).

2.4.3 Near-infrared spectroscopy

According to the American Society of Testing and Materials (ASTM), NIR spectroscopy covers the electromagnetic spectrum between 780 and 2526 nm ($12\,820 - 3959\text{ cm}^{-1}$). The peaks observed in NIR spectra are mostly due to the absorption bands originating from overtones and combinations of the fundamental modes of $-\text{CH}$, $-\text{NH}$, $-\text{OH}$ (and $-\text{SH}$) functional groups (Fig. 5, Siesler, Ozaki et al. 2002). NIR spectroscopy reveals information about the physical (particle size, density, morphology, temperature) and also chemical properties (vibrations connected with hydrogen bonding) of the sample (Reich 2005). Thus the information is obtained mostly from the intramolecular vibration changes in the crystal.

Conversely to Raman, NIR spectroscopy is extremely sensitive towards water. Water gives the strongest absorption bands approximately at 1940 (5150 cm^{-1}) and 1450 nm (6900 cm^{-1}). NIR spectroscopy enables differentiation between free water and structural water. The shift to

higher wavelength occurs with hydrogen bonded water (Räsänen, Rantanen et al. 2001). Based on the sensitivity towards water, hydrates are usually easily distinguished from anhydrous forms.

The absorption bands in NIR spectra are broad, overlapping and relatively weak compared to fundamental modes in MIR spectra. Therefore, NIR spectroscopy requires the use in combination with multivariate analysis methods, which extract the relevant information from the spectra (Stephenson, Forbes et al. 2001). Due to lower sensitivity the NIR spectroscopy measurements suit more for the analysis of major components.

NIR spectroscopy measurements are performed either in diffuse reflectance or transmission mode, or in combination of the two - transflection mode. Diffuse reflectance is more widely used for solid-state analysis (Lodder and Hieftje 1988), but problems associated with quantitative assessment of bulk solid dosage forms, such as inhomogeneity of the sample and the physical properties of sample, have increased the interest to use transmission mode. There are also complications with transmission measurements, such as relatively small wavelength region available for analysis related to absorption coefficient of the material (Gottfries, Depui et al. 1996). However, transmission mode NIR spectroscopy is useful for intact tablet assessment (Lodder and Hieftje 1988; Abrahamsson, Johansson et al. 2005). The concentration of paracetamol and metoprolol in intact tablets has been successfully evaluated by transmission NIR spectroscopy (Gottfries, Depui et al. 1996; Eustaquio, Blanco et al. 1999).

Similarly to Raman, NIR spectroscopy allows rapid, non-destructive and non-invasive measurements, and can be used for process monitoring and qualitative and quantitative analysis. Hence NIR spectroscopy is especially useful for monitoring the changes in hydration state of an API throughout the manufacturing processes (Higgins, Arrivo et al. 2003). A comprehensive review about the NIR spectroscopy applications in pharmaceutical industry has been reported by Blanco et al. (Blanco, Coello et al. 1998). As with Raman, NIR spectroscopy can be used to determine the homogeneity of the powder blend using an on-line measurement (Sekulic, Ward et al. 1996). Monitoring of the hydrate formation during the high shear granulation (Rantanen, Wikström et al. 2005b) and wet granulation processes has been reported (Jørgensen, Luukkonen et al. 2004). Thus in combination with multivariate data analysis, NIR spectroscopy can be applied for determination of the process end-points and detecting outliers during production (Rantanen, Wikström et al. 2005b). In-line NIR spectroscopy has been applied for monitoring the drying phase of a wet granulation process (Davis, Peck et al. 2004).

2.4.4 Terahertz pulsed spectroscopy

Terahertz radiation lies between the microwave and infrared regions, as part of the far-infrared region, between 130 and 2 cm^{-1} . TPS detects low frequency molecular flexing and intermolecular vibrations in the solid state. In crystals, phonon modes are also detected and therefore, the information is largely gathered from the intermolecular level (Taday and Newnham 2004) and thus crystal lattice changes are directly probed (Day, Zeitler et al. 2006). In addition to the solid state, TPS reveals information about the molecular rotations in the gaseous phase.

In TPS, terahertz radiation is commonly generated using femtosecond laser pulses which excite photoconductive switches (Taday and Newnham 2004). TPS measurements can be performed in transmission mode, which usually requires compact preparation with an appropriate diluent (usually polyethylene and polytherafluoroethylene powders are used). Alternatively, TPS measurements can be conducted using specular reflectance or an ATR crystal, and therefore no sample preparation is needed.

The peaks in TPS spectra cannot easily be assigned at present and thus the interpretation of spectra is more difficult compared to MIR spectroscopy. One reason is that the information about the intra- and intermolecular vibrations are both represented in TPS spectra, which complicates interpretation. Another reason is that historically the terahertz region has been much less used than the MIR region to study the pharmaceutical materials. However, harmonic rigid molecule lattice dynamics and density functional theory calculations have been successfully performed for some pharmaceutical compounds to reveal some information behind the modes (Day, Zeitler et al. 2006).

Two review articles have recently been published that cover the applications of TPS in pharmaceutical research (Ho, Zeitler et al. 2006; Zeitler, Taday et al. 2007). Similarly to Raman and NIR spectroscopy, TPS is a fast and non-destructive technique. It can be used for monitoring phase transitions at variable temperatures. A phase transition of carbamazepine from form III to form I could be monitored upon heating using variable temperature TPS (Zeitler, Newnham et al. 2005). The dehydration of glucose-D-monohydrate has also been monitored and the kinetics assessed (Zhang and Liu 2006). In addition, polymorphs and crystallinity can be quantified with TPS (Strachan, Taday et al. 2005).

2.5 *Data analysis*

During process monitoring, vibrational spectroscopy techniques are usually combined with specific data analysis methods, which provide either qualitative (e.g. classification, clustering) or quantitative information about the process. Since spectroscopy provides large data-sets, the extraction of important and meaningful information facilitates the efficient process overview and deeper understanding.

The multidimensional data can be analysed by various multivariate analysis methods, with principal component analysis (PCA) and partial least squares (PLS) regression being the most widely used linear methods for pharmaceutical process monitoring (Pöllänen, Häkkinen et al. 2005; Rantanen, Wikström et al. 2005b; De Beer, Baeyens et al. 2006; Jørgensen, Miroshnyk et al. 2006). These two methods have proved suitable for analysing process data largely due to the ability to cope with dimensionality, collinearity, noise and missing data (Eriksson, Johansson et al. 2001). The multivariate analysis methods used in this thesis, namely PCA, PLS regression and PLS discriminant analysis (PLS-DA), are discussed in more detail.

2.5.1 **Principal component analysis**

In an early stage of data analysis, when a relatively new problem is investigated, then PCA is often performed (Haaland and Thomas 1988; Eriksson, Johansson et al. 2001). This method is suitable for data overview and classification purposes. PCA is usually performed for qualitative investigations, where the scores and loadings are projections of the X-matrix, thus only the X-matrix is considered (*Fig. 6*).

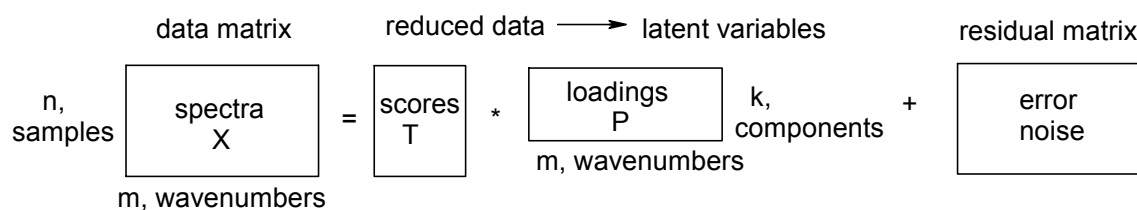


Figure 6. PCA projects the meaningful information into the scores and loading plots and reduces the data matrix (modified from Geladi 2003).

Consequently, the score values will provide information about the similarities and differences observed in the spectra, and the monitoring of the process can be performed using score values

over time. The loadings explain the origin of scores, and hence their relationship to the original spectra.

2.5.2 Partial least squares regression

PLS regression can be used for quantitative analysis. PLS regression is a projection method relating two data matrices, X and Y, to each other by a linear multivariate model (Haaland and Thomas 1988; Krzanowski and Marriot 1995; Eriksson, Johansson et al. 2001; Wold, Sjöström et al. 2001)(Fig. 7). It is used for multivariate calibration, quantitative structure-activity relationships modelling and for process modelling and optimisation. PLS regression uses latent variables analysis to compress the size of spectra and remove any insignificant information. The concentration information together with spectral variance are both used in the compression process and included in the latent variables that are correlated with concentration (Pelletier 2003). Weights and regression coefficients in PLS allow the interpretation of the model, reveal how X (the spectra) and Y (the concentration) are related. The prediction of a new sample Y_{pr} can be performed, when the relation is developed in a calibration model (Fig. 7).

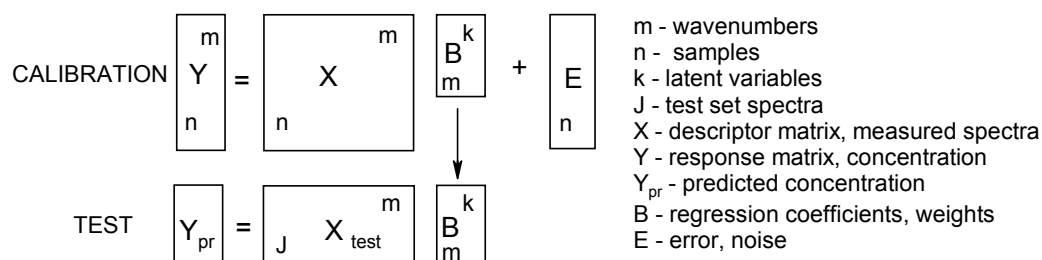


Figure 7. PLS regression, calibration is performed to find B, which works well in calibration set; B is used with test set spectra to calculate the predicted concentration Y_{pr} (modified from Geladi 2003).

2.5.3 Partial least squares discriminant analysis

PLS-DA can be considered as an extension of the projection methods and it is based on PLS technique. Similarly to PCA, this analysis method is used to qualitatively discriminate between different classes of observations on the basis of their X-variables, thus each class membership is known and taken into account. PLS-DA enables the rotation of the projection to give latent variables that focus specifically on the class separation (Eriksson, Johansson et al. 2001), and the

covariance between the scores (and weights) of the X and Y matrices is maximised. Therefore also Y-matrix contribution is taken into account and this refers more to the variation of the constituent in interest.

3 AIMS OF THE STUDY

The overall aim of the present thesis was to increase the molecular level understanding of the dehydration behaviour of pharmaceutical hydrates using vibrational spectroscopy (NIR, Raman, and terahertz pulsed spectroscopy) combined with multivariate data analysis. The phase transitions were examined firstly *in situ*, and secondly during a pharmaceutical unit operation - fluidised bed drying.

Using diverse pharmaceutical hydrates as model APIs, the specific aims were:

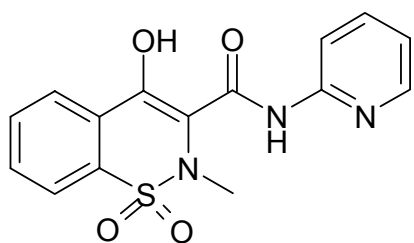
- To investigate the differences between the NIR, Raman and terahertz spectra of anhydrous and hydrate forms of model APIs (**I - V**)
- To qualitatively monitor the solid-state changes of pharmaceutical hydrates during isothermal dehydration using NIR, Raman and TPS spectroscopy in combination with principal component analysis (PCA)(**II, III**) or partial least squares discriminant analysis (PLS-DA)(**I**)
- To assess and compare the suitability of different spectroscopic techniques to examine dehydration from compacts and investigate the effect of sample preparation (mixed, surface layer and middle layer compacts) on the dehydration behaviour of hydrates (**III**).
- To quantify the solid-state forms during the isothermal dehydration at different temperatures using *in situ* NIR and Raman spectroscopy combined with partial least squares (PLS) regression (**V**).
- To investigate and quantify the solid-state forms that appear during isothermal dehydration of hydrate granules in fluidised bed dryer using NIR (**IV**) and Raman spectroscopy (**IV, V**) together with PLS regression.

4 EXPERIMENTAL

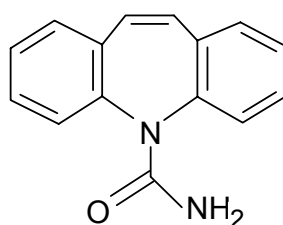
A more detailed description of materials and methods is given in the original publications which are referred to by their respective Roman numerals (**I - V**).

4.1 *Materials*

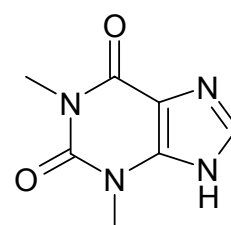
Raw materials of anhydrous piroxicam (PRX, **I, II, III, V**) and carbamazepine (CBZ, **I, II, V**) were purchased from Hawkins, Inc. (Minneapolis, USA). Theophylline (TP, **II, IV**) was obtained from BASF (Ludwigshafen, Germany). Hydrate forms of PRX, CBZ, and TP were prepared by recrystallisation from hot saturated aqueous solutions, which were slowly cooled to room temperature. PRX form I (PRXAH), CBZ form III (CBZF3), and TP anhydrate were produced by heating the hydrate crystals under reduced pressure (72 mBar) at 373 K for 24 hours. Anhydrous CBZ form I (CBZF1) was obtained by heating the raw CBZF3 at 443 K and normal pressure for two hours according to the method described by Lefebvre et al. (Lefebvre, Guyot-Hermann et al. 1987). Amorphous CBZ (CBZA) was produced by quench cooling of the melt using liquid nitrogen.



PIROXICAM



CARBAMAZEPINE



THEOPHYLLINE

4.1.1 Preparation of granules (**IV, V**)

TP raw material was granulated with purified water in a planetary mixer during which a solvent mediated phase transition resulted in formation of TP monohydrate (TPMH) granules. The sieve fraction between 1 and 2 mm was used for fluidisation experiments (**IV**). Due to the difficulty of obtaining CBZ dihydrate (CBZDH) granules by wet granulation (Otsuka, Hasegawa et al. 1999), the crystallised CBZDH powder was mixed with purified water in a mortar, and pure CBZDH

granules were formed. The sieve fraction between 1.8 and 2.8 mm was subjected to fluidised bed experiments (V).

4.1.2 Preparation of compacts (II, III)

Either polyethylene (PE, Induchem, Volketswil, Switzerland)(III) or polytetrafluoroethylene (PTFE, Aldrich, UK)(II, III) powders were used to prepare compacts. API powder (approximately 15 mg) was mixed together with the polymer (1112 mg), and compacts with 13 mm diameter were pressed using two ton compression for two minutes. The compacts were approximately 3.5 mm thick. PE was used as diluent for room temperature TPS spectra collection, and PTFE was chosen for *in situ* dehydration measurements at higher temperatures. Different compacts were prepared to investigate the effect of sample preparation on dehydration behaviour of the APIs (III). Accordingly, for mixed layer samples, PTFE was mixed with API, surface layer samples, where the API layer was on the surface of PTFE and middle layer samples, where the API layer was in between two PTFE layers, were prepared.

4.2 Analytical methods

4.2.1 Reference methods

4.2.1.1 X-ray powder diffraction (I – V)

Crystal structures were verified by X-ray powder diffraction (XRPD) using a theta-theta diffractometer (D8 Advance, Bruker AXS GmbH, Karlsruhe, Germany) and comparing the experimental results to the theoretical patterns in the Cambridge Structural Database (CSD (Beebe, Pell et al. 1998). Variable temperature XRPD (VT-XRPD) patterns were measured using the same XRPD diffractometer. VT-XRPD scans were obtained at different temperatures: CBZ from 298 K to 373 K and PRX from 298 K to 443 K (I). Isothermal XRPD experiments were carried out to verify the solid-state forms during isothermal heating and confirm the spectroscopy results. Experiments were performed at two temperatures for PRX monohydrate (PRXMH)(at 381 and 405 K) and at three temperatures for CBZDH (at 313, 323 and 338 K, V). VT-XRPD from PRXMH surface layer compacts was performed from 303 to 447 K. The compacts were fitted into an XRPD sample holder by removing the edges and some of the PTFE from the compacts (III).

4.2.1.2 **Differential scanning calorimetry (I, IV)**

Differential scanning calorimetry (DSC) was performed with a TA Instruments 910S differential scanning calorimeter (TA Instruments Inc., New Castle, DE, USA). The DSC system was calibrated using indium (mp 429.85 K) and benzophenone (mp 320.80 K) standards. Samples were analysed using open aluminium pans at a heating rate of 10 K min⁻¹.

4.2.1.3 **Thermogravimetric analysis (I, III, V)**

A Mettler Toledo TGA 850 system was used for the thermogravimetric analysis (TGA, Mettler Toledo, Inc., Greifensee, Switzerland). TGA experiments were performed in open aluminium oxide (Al₂O₃) pans (70 µL). Non-isothermal TGA was carried out under nitrogen purge (balance purge, 50 mL min⁻¹) at a heating rate of 10 K min⁻¹ (I). Direct isothermal TGA measurements were performed without nitrogen purging and the sample was inserted after the set-temperature was reached to keep the measurement parameters close to the hot-stage spectroscopy conditions (V). Non-isothermal TGA was performed with compacts under nitrogen purge (balance purge, 50 mL min⁻¹) with a heating rate of 5 K min⁻¹ (III). The compacts were fitted into the sample holder by removing the PTFE part and the edges, and approximately 10 mg of sample was used.

4.2.1.4 **Karl Fischer titrimetry (I, IV, V)**

A Karl Fischer titrator (Mettler DL 35, Switzerland) was used to verify the water content (expressed as % w/w) of all the solid-state forms. The standard sample for calibration was analytical grade sodium tartrate hydrate (Hydranal®, Sigma-Aldrich Finland Oy, Helsinki, Finland; water content 15.7% w/w).

4.2.1.5 **Optical microscopy (I)**

Particle morphology of the hydrate forms was investigated with optical microscopy (DAS Mikroskop LEICA DM LB, Leica Mikroskopie and Systeme GmbH, Wetzlar, Germany).

4.2.2 Spectroscopy

4.2.2.1 Near-infrared spectroscopy (I, III, IV, V)

NIR spectra were recorded with a NIR spectrometer (NIR-256L-2.2T2, Control Development Inc., South Bend, IN, USA) having a thermoelectrically cooled 256 element InGaAs array detector, tungsten light source and using fiber optic reflectance probe (six illuminating optical fibers around one signal collecting fiber). The power of the light source leaving the excitation fibers was approximately 7.5 mW. A reference spectrum was recorded with a Teflon background. The spectra were recorded from 1100 to 2200 nm (9091 - 4545 cm^{-1}) with a 15 ms integration time and the number of averaged scans per spectrum was 16. The resolution for the NIR system was approximately 8 nm.

4.2.2.2 Raman spectroscopy (I, III, IV, V)

Raman spectra were collected using a Raman spectrometer (Control Development Inc., South Bend, IN, USA) equipped with a thermoelectrically cooled CCD detector (1024x64) and a fiber optic probe (RamanProbe™ RPS785/12-5, InPhotonics, Norwood, MA, USA). The laser source was an enhanced diode laser system (Starbright 785S, Torsana Laser Technologies, Skodsborg, Denmark), which operated at 785 nm. The laser power was 500 mW. Spectra were recorded from 2200 to 200 cm^{-1} with a 1 s integration time and each spectrum was the average of 5 scans. Cyclohexane was used to calibrate the Raman shift. The resolution for the Raman systems was approximately 8 cm^{-1} . All NIR and Raman spectra were collected every 10 s (I, V) or 15 s (III, IV) using CDI Spec32 software (Control Development Inc., South Bend, IN, USA). No purging gas was used for NIR and Raman spectroscopy experiments covered in papers I and V. All NIR and Raman spectra from PRX compacts were collected under dry nitrogen purge (flow rate 5 L min^{-1})(III).

4.2.2.3 Terahertz pulsed spectroscopy (II, III)

TPS spectra were recorded in transmission mode using a TPI spectra1000V spectrometer (TeraView Ltd, Cambridge, UK) with a resolution of approximately 1 cm^{-1} . Spectra were recorded by co-adding 450 scans (15 s acquisition time) over the range 115 - 2 cm^{-1} . To prevent interference of water vapour in the terahertz spectra, the sample chamber was purged with dry nitrogen prior to and throughout the experiment (flow rate 5 L min^{-1}). Pure PE or PTFE compacts

(1100 mg) were used as references. Sample and reference spectra were calculated by fast Fourier transformation of the time domain waveforms using Blackmann-Harris three-term apodisation and absorbance spectra were calculated from the sample and reference spectra (Taday and Newnham 2004). TPI Spectra software (TeraView Ltd, Cambridge, UK) was used for TPS measurements and spectrum processing.

4.3 *Hot-stage spectroscopy*

4.3.1 **Hot-stage near-infrared and Raman spectroscopy (I, III, V)**

The heating device used for NIR and Raman spectroscopy experiments was a Mettler Toledo FP 900 Thermosystem (Mettler Toledo AG, Greifensee, Switzerland) comprised of a Mettler Toledo FP 90 Central processor and a modified Mettler Toledo FP82 HT microscope hot stage (**I, III, V**). The powder (approximately 10 mg) was mounted on a microscope slide. The heating was initiated after the lag-time to isothermal set-temperature (**I**) or after the set-temperature was reached (**V**). The compact was mounted in an aluminium holder on the open hot-stage, and covered by three washers and two steel washers (**III**). The temperature on the compact surface was checked using an infrared thermometer (KM814, Comark Limited Inc., Stevenage, Hertfordshire, UK)(**III**). Reproducibility of the measurements was verified using duplicate (**I, V**) or triplicate experiments (**III, V**). The relative standard deviations (RSD) were calculated from the model predictions (PLS regression)(**IV, V**) or using score values (PCA, PLS-DA)(**I, II, III**).

4.3.2 **Hot-stage terahertz pulsed spectroscopy (II, III)**

TPS experiments were performed employing a heatable transmission cell with no windows (Specac, Orphington, UK) and the temperature was controlled using a 3000 series high stability controller (Specac, Orphington, UK)(Zeitler, Newnham et al. 2005)(**III, V**). The sample cell as such consisted of a copper block with a 13 mm aperture on one side and an 8 mm aperture to the other side enclosed by a standard Specac variable temperature cell. The variable temperature cell was placed at the focal point of the terahertz optics and the sample chamber was sealed during purging with dry nitrogen gas. Isothermal dehydration experiments were carried out at 405 K (**III**), non-isothermal measurements were performed with the heating rate of 5 K min⁻¹(**II, III**). To evaluate the reproducibility of the measurements the RSD of the PC1 scores were calculated (**III**).

4.4 *Fluidised bed drying (IV, V)*

TPMH (batch size 4.5 g)(**IV**) and CBZDH granules (batch size 1.94 g)(**V**) were dried in a microscale multichamber fluidised bed dryer (MMFD, Ariacon Oy, Turku, Finland, Räsänen, Rantanen et al. 2003; Räsänen, Rantanen et al. 2004) at different temperatures with an air flow of 215 and 260 mL s⁻¹, for TPMH and CBZDH respectively. Experiments were performed in triplicate to confirm the reproducibility, the RSD values were calculated from Raman spectroscopy PLS model predictions. Prior to the experiment the fluidisation chamber was pre-heated to the set-temperature. In-line spectroscopic measurements were performed with NIR and Raman probes through a quartz sight glass window (**IV, V**).

4.5 *Data analysis*

Multivariate data analysis was used for the investigation of dehydration and was performed using PCA (**II, III**), PLS-DA (**I**), and PLS regression (**IV, V**) Data were processed using Simca-P (Version 10.5, Umetrics AB, Umeå, Sweden) and Matlab (Version 6.5, The Mathworks Inc., South Natick, MA).

To remove noise from spectral data, pre-processing was performed. Several pre-processing methods were tested for NIR and Raman spectral data; the best results were obtained with standard normal variate (SNV) transformation (Barnes, Dhanoa et al. 1989). Mean centering (**I, III, V**) and scaling to unit variance (**IV**) were also performed. All NIR, Raman and TPS spectra were normalised and scaled so that their relative intensities matched those of the intensity ratio in the raw spectra (**III**).

Several parameters, such as accuracy, precision, specificity, linearity, robustness of an assay, are inherent to quantitative method development (ICH 1995). Therefore, these were all considered and/or verified during the model construction phase. Spectral ranges were selected for analysis and model outliers identified and removed to develop models with the best quality. Specific model parameter values (root mean square error of prediction (RMSEP), root mean square error of calibration (RMSEC), goodness of fit (R^2), goodness of prediction (Q^2)) revealed the model quality and performance. Accordingly, the PLS-DA, PCA and PLS regression models consisted of the regions where the largest differences between the forms were seen. For Raman spectroscopy

models, the effect of fluorescence on Raman spectra was reduced by careful wavenumber selection. External test-set validation was used to evaluate model performance.

4.5.1 Principal component analysis (II, III)

PCA models were developed from dehydration data that enabled monitoring of the dehydration behaviour of TP (II) and PRX hydrates (III). Principal components (PCs), expressed by the scores and loadings plots, were used to represent and understand the spectral variation. This is an easy way to interpret the multivariate spectral information, because two or three dimensional figures are produced from the multivariate systems. These plots helped to reveal the largest variations in spectra and identify the origin of these spectral differences. Therefore, the score values in relation to time or temperature revealed the dehydration behaviour together with the dehydration temperature ranges.

4.5.2 Partial least squares discriminant analysis (I)

PLS-DA models were constructed that consisted of spectra of pure solid-state forms of PRX and CBZ (I). For PRX two forms were included, PRXMH and PRXAH, since it was known that PRXMH dehydrates only to PRXAH (Sheth, Zhou et al. 2004). All the forms that appeared during CBZDH dehydration by VT-XRPD were used for CBZ model construction: CBZDH, CBZF3, CBZF1 and CBZA. Similarly to PCA models, the PLS-DA applied all the spectral information and developed scores and weights (similar to the loadings in PCA) plots. The origin of the spectral variation was directly related to the different solid-state forms. Therefore the model could classify the forms and cluster them in the scores plot. When the dehydration data were projected on the scores plot, the dehydration could be followed from one form to another, and in addition multiple solid-state transitions were revealed.

4.5.3 Partial least squares regression (IV, V)

4.5.3.1 Preparation of calibration models (IV, V)

Calibration models were constructed for PRX, CBZ and TP. Binary mixtures were prepared and the NIR and the Raman spectra collected for PRX and TP consisting of the PRXMH and PRXAH

(**V**), and TPMH and TP anhydrate (**IV**). The concentration range was between 1 and 100%. Quaternary mixture design, developed in Modde (Version 10.5, Umetrics AB, Umeå, Sweden), was used for CBZ calibration model construction (**V**). The model consisted of CBZDH, CBZF3, CBZF1 and CBZA in different ratios. The calibration model spectra were collected under similar conditions to the *in situ* dehydration experiments. The sample was placed on a microscope slide; a rotating accessory was used for calibration model spectra collection to ensure representative sampling of the overall composition (**I**, **IV**, **V**). In addition to room temperature spectra, the spectra at dehydration temperatures for solid-state forms were added to the PRX and CBZ calibration models to incorporate temperature induced variation in the model. CBZDH spectra were not collected at dehydration temperatures due to rapid transformation (**V**).

Calibration model spectra were used to build the PLS regression models (**V**, Table 1 for PRX, and Table 2 for CBZ). In case of PLS regression the differences found in spectra were explained by the latent variables (similar to PCs in PCA), which were visualised by the scores and weights plots. The dehydration data were projected into the PLS regression model, which then predicted the contents of solid-state forms during and after the *in situ* and real time dehydration.

5 RESULTS AND DISCUSSION

A deeper understanding of dehydration and multiple solid-state transformations was obtained through process monitoring by vibrational spectroscopy. This section is divided into different parts, which are written as shown in *Figure 8*.

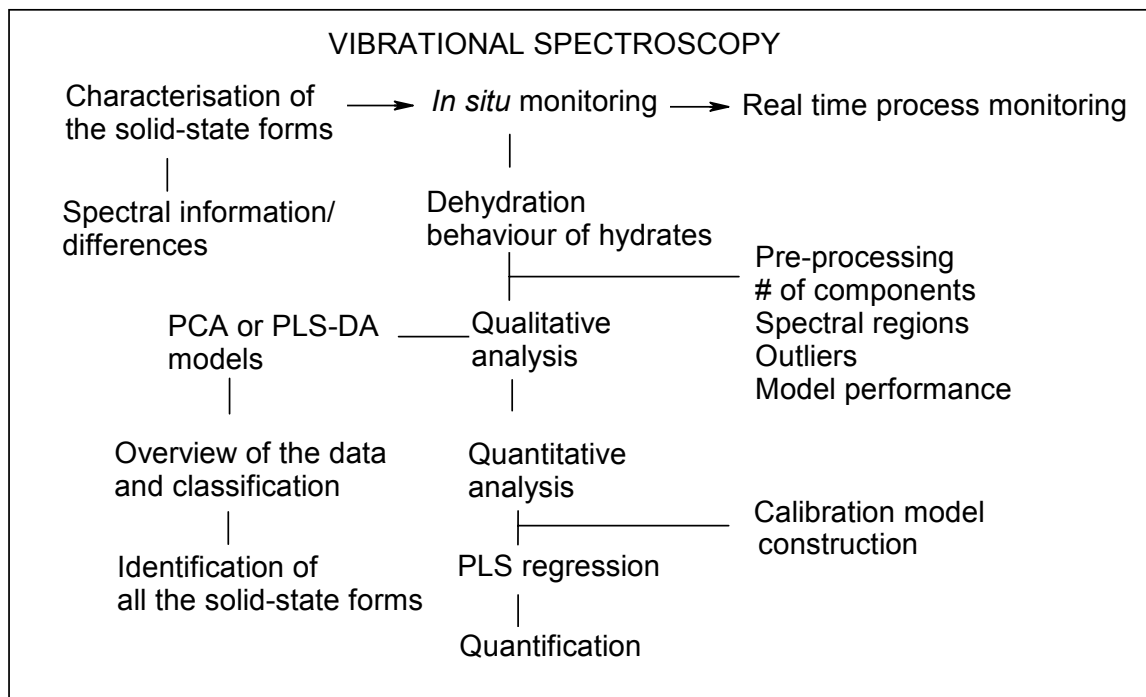


Figure 8. General approach used to investigate the dehydration behaviour of pharmaceutical hydrates using vibrational spectroscopy.

Diverse pharmaceutical hydrates were selected as model APIs, to develop a method that can be used to qualitatively and quantitatively investigate their dehydration behaviour. This method serves as a model approach that could be used further for in-line process monitoring and control purposes, and is not necessarily limited to these model APIs. Furthermore, the selection of the technique and the analysis method (univariate versus multivariate) depend on the process and APIs under investigation.

At first the physical characterisation of possible solid-state forms was conducted with spectroscopy and reference methods. Secondly, the dehydration behaviour was monitored *in situ* using spectroscopic techniques together with multivariate analysis. This combination revealed the possible solid-state forms and consequently allowed the quantification of the forms. The

spectral data were visualised in score and loading (or weight) plots. Reference methods were used to confirm the spectroscopy results. Once the data for the dehydration behaviour was obtained *in situ*, real time process monitoring was performed during unit operation – fluidised bed drying. More detailed results and discussion can be found from the original papers (**I - V**).

5.1 **Characterisation of pharmaceutical hydrates**

All of the model substances exhibit polymorphism and have one known hydrate form. PRX, a non-steroidal anti-inflammatory drug, can form a monohydrate and it has three anhydrous forms (PRXAH, forms II, and III, Vrečer, Urbinc et al. 2003; Sheth, Bates et al. 2004). CBZ, has four reported anhydrous forms (CBZF1 (stable at high temperature), form II, CBZF3 (stable at room temperature), and form IV), and in aqueous solution/suspension and under high humidity it transforms to CBZDH (Kahela, Aaltonen et al. 1983; Kaneniwa, Yamaguchi et al. 1984; Grzesiak, Lang et al. 2003). For PRX and CBZ, in addition, the amorphous form can be obtained by different methods (Li, Han et al. 2000; Sheth, Bates et al. 2004). TP exhibits two enantiotropic polymorphs, with form I stable at high temperatures and form II stable at room temperature (Burger and Ramberger 1979b; Suzuki, Shimomura et al. 1989), and as mentioned in the introduction section the anhydrous TP forms can easily form a monohydrate in aqueous environments and under high humidity (Shefter and Highuchi 1963; Herman, Remon et al. 1988; Ando, Ishii et al. 1992). In addition, TP has another metastable anhydrous form (Fokkens, van Amelsfoort et al. 1983). All anhydrous and hydrate forms of model substances were verified by XRPD analysis. The water contents were confirmed by TGA and Karl Fischer titrimetry. The water contents of anhydrous forms were all within the limits set in Ph. Eur. (below 0.5% w/w), for hydrate forms the water contents were in a reasonable agreement with the theoretical water contents of 5.2%, 13.2% and 9.1% w/w, for PRXMH, CBZDH and TPMH, respectively.

5.1.1 **Spectroscopy for identification of the solid-state forms**

In addition to XRPD, NIR and Raman spectroscopy, and TPS could be used to distinguish between the anhydrous TP form II and TPMH (**II**, Fig. 1, **IV**, Fig. 4), and all three spectroscopic methods were able to differentiate between PRXMH and PRXAH (**I**, **II**, **III**, **V**). As can be seen from *Figure 9*, the spectra of two forms of PRX revealed relatively large differences, and consequently their identification was possible with all techniques.

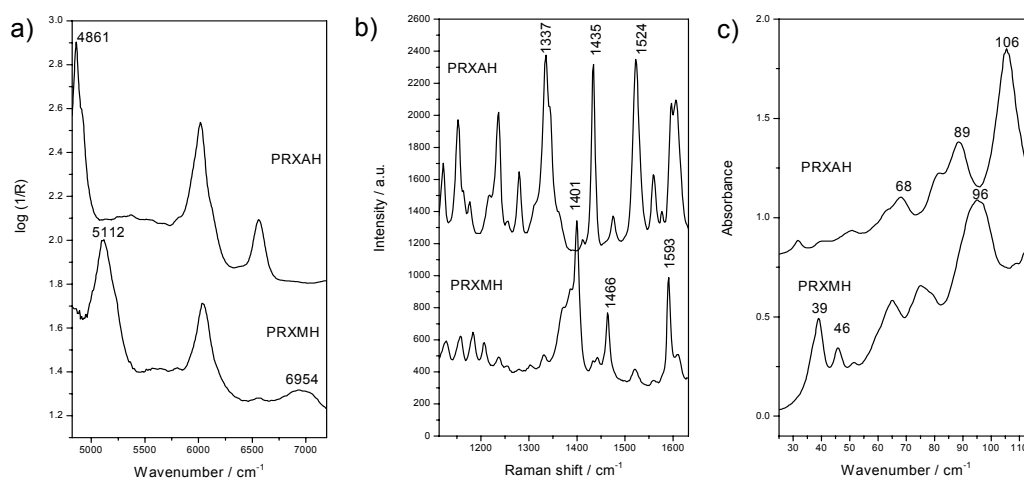


Figure 9. PRXMH and PRXAH spectra using a) NIR spectroscopy, b) Raman spectroscopy, and c) TPS.

With CBZ, NIR and Raman spectra revealed subtle differences between CBZF3, CBZF1, CBZA and CBZDH (**I**, Fig. 6; **V**). However, it was possible to distinguish between the forms. Previous studies have reported that CBZF3 and CBZF1 obtain also different TPS spectra (Strachan, Rades et al. 2004), which allow separation of the forms and monitoring of the phase transformation (Zeitler, Newnham et al. 2005). In addition, in this thesis it was found that with TPS anhydrous CBZF3 and CBZDH could be distinguished (**II**).

The differences seen in the spectra of different solid-state forms were the key factors that allowed the process monitoring during the phase transition. It is important to reveal the origin of the spectral features; only then can the molecular level information be determined to improve the overall understanding of the process. Therefore the spectral features were identified by assigning of the peaks based on the information obtained from the literature (**I**, **IV**, **V**; **III**, Table 1).

NIR spectroscopy is known to be more sensitive towards water and O-H, N-H, C-H, S-H bonds. This can be considered an advantage when hydrates are investigated. The main differences between anhydrate and hydrate forms were located at the wavelengths where the absorption of lattice-bound water occurred. The combination band related to O-H stretching and the first overtone of water at 1940 (5150 cm^{-1}) and 1450 nm (6900 cm^{-1}), respectively, revealed the presence of hydrate forms, and these regions were used to conduct qualitative and quantitative analysis.

Raman spectroscopy detected intramolecular vibrations and was sensitive towards conformational changes. The finger-print region (below 1500 up to 1700 cm^{-1}) contained the largest variation between the forms, mainly comprising the C-C, C-N, and aromatic ring vibrations. TPS directly probed the changes in crystal lattice, and thus the changes in crystal structure (unit cell dimensions) provided a basis for differentiation between all the solid-state forms. The peaks in TPS spectra are due to the intermolecular phonon modes, but the peak assignment was not performed due to a lack of available information on the origin of these modes. For every model drug, the regions with the largest differences were used for further analysis.

5.2 *Monitoring dehydration in situ*

The dehydration behaviour of PRXMH (**I**, **III**, **V**), CBZDH (**I**, **V**) and TPMH (**II**, **IV**) investigated by spectroscopy is explained in separate paragraphs below. In addition, DSC, TGA, and VT-XRPD revealed the dehydration temperature ranges and verified the spectroscopy results (**III**, **V**).

5.2.1 **Piroxicam (I, III, V)**

5.2.1.1 **Qualitative investigation of dehydration (I)**

According to DSC and TGA the dehydration temperature was between 363 and 423 K, consistent with the literature (Vrečer, Vrbinc et al. 2003). These results were verified by VT-XRPD (**I**). Previously, thermal methods have shown that dehydration kinetics and the behaviour of PRXMH can be investigated (Sheth, Zhou et al. 2004). However, the suitability of vibrational spectroscopy to monitor isothermal dehydration has not previously been shown. The results from this thesis reveal that NIR and Raman spectroscopy and PLS-DA modelling allowed monitoring of the isothermal solid-state transition from PRXMH to PRXAH.

For both NIR and Raman spectroscopy PLS-DA models, the first component incorporated the largest differences in spectra due to the solid-state forms of PRX. Therefore, the dehydration could be followed using the first component versus time plots (**I**, Fig. 4). This plot revealed the end-point of dehydration. At 381 K the dehydration lasted approximately 75 min, but at 405 K it lasted only approximately 6 min. The dehydration profiles obtained with both NIR and Raman spectroscopy were in a good agreement with each other.

The spectral information enabled prediction of the dehydration behaviour of PRXMH. Although, PRX has also two other anhydrous forms besides PRXAH, the isothermal dehydration on hot-stage at variable temperatures produced only PRXAH. These results were in good agreement with a previously reported study (Sheth, Zhou et al. 2004).

5.2.1.2 Quantitative investigation using NIR and Raman spectroscopy (V)

Once the qualitative information was obtained about the isothermal dehydration of PRXMH, it was of interest to also conduct quantitative analysis. Monitoring the amount of solid-state forms at different temperatures and time-points allows implementation of sufficient control on the solid-state transformations under different conditions.

PLS regression models revealed that the two solid-state forms involved with dehydration of PRXMH can be quantified using both NIR and Raman spectroscopy (V, Fig. 2). The calibration models and the PLS regression model predictions were improved when the variability connected with temperature was included in the model. Similarly to the PLS-DA models, the first latent variable differentiated between PRXMH and PRXAH forms and the second and, in case of Raman spectroscopy, the third latent variables explained the temperature effects.

The results from spectroscopic measurements were in good agreement with the isothermal TGA and XRPD results. Similar profiles obtained using NIR and Raman spectroscopy show that the changes in crystal structure of PRXMH and in water content occur simultaneously as previously reported (Sheth, Zhou et al. 2004). Sheth et al. has reported that the dehydration behaviour of PRXMH is largely affected by the hydrogen bonding network between PRX-PRX, PRX-water and water-water molecules and the dehydration follows the two-dimensional phase boundary model. Thus a direct solid-state transformation occurs from PRXMH to PRXAH, with no intermediate forms. When different temperatures were used for dehydration, it was confirmed that the amount of PRXMH reached a plateau faster at 405 K compared to 381 K.

5.2.1.3 Dehydration from compacts using NIR, Raman and TPS (III)

Generally, dehydration is investigated with powders. However, in several occasions during manufacturing of solid dosage forms, powders are granulated or compacted (e.g. during tableting and granulation). This physical matrix consisting of API and excipients, may

considerably change the dehydration behaviour of APIs. And therefore, it was of interest to reveal the behaviour also under those conditions.

The effect of sample preparation on the dehydration behaviour from compacts was revealed using *in situ* TPS (III, Fig. 3), because Raman and NIR spectroscopy were found to be limited by their small sampling volume and interference from the polymer matrix. Isothermal dehydration of PRXMH at 405 K from compacts, in which PRXMH was dispersed throughout the compact, deposited on one face of the compact, or included as a layer within the compact, showed that the sample preparation affected the dehydration kinetics. The dehydration with surface layer samples was complete after 18 min, however, the mixed layer samples were not completely transformed to PRXAH. In the middle layer samples, the dehydration had not even begun during the same period. This can be explained by the fact that the crystal water has to diffuse out of the polymer matrix on the surface of the compact. The spectral changes for middle layer samples were the effect of temperature on the TPS spectra, as the sample was heated to 405 K, a phenomenon that has been previously reported (Zeitler, Newnham et al. 2005).

In addition, Raman and NIR spectroscopy and TPS revealed the non-isothermal dehydration behaviour of PRXMH from the surface layer compacts. PCA of spectroscopy and XRPD data and the resulting PC scores and loadings were used to monitor the dehydration. The first PC (PC1) distinguished between the two solid-state forms of PRX, and the second PC (PC2) mainly explained peak broadening and peak shifting due to temperature effects. The PC1 versus temperature plots facilitated comparison between the techniques. The dehydration profiles obtained using TPS, Raman and VT-XRPD were similar due to their sensitivity to structural information. TGA and NIR spectroscopy profiles showed a similar trend, which proves their high sensitivity towards water content. The dehydration temperature range was the lowest with TPS, intermediate for Raman spectroscopy and highest for NIR spectroscopy (III, Fig. 5). The apparent dehydration temperatures varied due to the fact that crystal water has to diffuse out from the compact for dehydration to occur. During this process the water stayed within and on the surface of the compact as free water and after that the dehydration of the compact was completed. Because of this, the free water was also detected by NIR spectroscopy which had the highest dehydration temperature range.

5.2.2 Carbamazepine (I, V)

5.2.2.1 Qualitative investigation of dehydration (I)

For DSC analysis and TGA the dehydration temperature range was between 313 and 353 K, which is in good agreement with literature values (Krahn and Mielck 1987; Li, Han et al. 2000). The solid-state forms observed during non-isothermal and isothermal dehydration of CBZDH were also identified by XRPD. Isothermal TGA was also used as a reference method.

It has been reported that both non-isothermal and isothermal dehydration of CBZDH (prepared from CBZF3) largely depend on the humidity conditions around the sample (McMahon, Timmins et al. 1996; Han and Suryanarayanan 1997; Han and Suryanarayanan 1998). Under conditions where water cannot escape from around the sample, anhydrous CBZF3 is formed during non-isothermal dehydration, but when there is lower humidity CBZF1 is crystallised (McMahon, Timmins et al. 1996). Isothermal dehydration under low humidity produced CBZA (Han and Suryanarayanan 1998). When a constant water vapour pressure of 3.2 torr was used and temperature varied from 299 to 326 K, the dehydration resulted in CBZA at higher temperatures and in CBZF1 at lower temperatures (Han and Suryanarayanan 1998). Furthermore, an increase in water vapour pressure during dehydration induced the crystallisation of the product whereas no conversion was detected when the formed CBZA was exposed to high humidity after dehydration (Surana, Pyne et al. 2003). Therefore, not only the kinetics of transformation, but also the solid-state form obtained during heating is dependent on the humidity and temperature (Han and Suryanarayanan 1997; Han and Suryanarayanan 1998; Han, Zhang et al. 2003).

TGA provides the dehydration profiles at different temperatures, but no direct information about the solid-state form is obtained. XRPD on the other hand evaluates the solid-state forms, but not the water content. Using vibrational spectroscopy, it was possible to reveal both the dehydration kinetics and the solid-state form of the product at the same time. PLS-DA models helped to understand the dehydration behaviour of CBZDH. NIR spectroscopy is known to be very sensitive towards vibrations of O-H bands of water therefore the first component explained the dehydration by separating the CBZDH from other solid-state forms. Raman spectroscopy detects the structural and conformational changes, and thus the first component separated between the anhydrous CBZF3 and CBZF1. The second component represented the variations related with CBZDH, and the dehydration could be monitored using the second component versus time plots (I, Fig. 8).

It was seen from hot-stage spectroscopy results that the dehydration of CBZDH is more complicated than PRXMH dehydration behaviour. During solid-state transformation an intermediate lower crystallinity phase was identified, as has been previously reported (McMahon, Timmins et al. 1996; Han and Suryanarayanan 1997; Han and Suryanarayanan 1998) and the final isothermal dehydration product was a mixture of CBZ anhydrous forms. Thermodynamically, CBZF3 is the most stable form under dehydration temperatures used in this study (Krahn and Mielck 1987), and thus the formation of CBZF1 and CBZA during dehydration is due to the kinetic effects during dehydration. Both these solid-state forms are metastable under the experimental conditions, and they form together with the stable CBZF3. It appears that if the dehydration kinetics is fast enough, the crystal collapses during water removal and CBZA is formed under these conditions. If the temperature is too high, the molecular mobility is high enough to cause rapid nucleation and crystal growth.

The dehydration mechanisms are known to change depending on the temperature (Han and Suryanarayanan 1998). The experiments performed at three dehydration temperatures showed that the solid-state transformation kinetics changed. As expected, at 338 K the process was finished most rapidly, but at 313 K the dehydration lasted for approximately 150 min. More amorphous form and CBZF1 was produced during dehydration at 323 K.

5.2.2.2 Quantitative investigation using NIR and Raman spectroscopy (V)

To further investigate CBZDH dehydration behaviour, PLS regression modelling was performed for quantification of multiple solid-state forms. The appearance of CBZA during the dehydration and a mixture of CBZF3, CBZF1 and CBZA in the final product of dehydration demonstrate a need to quantitatively control the dehydration of CBZDH and subsequent solid-state forms. The stability of the final dehydration product is highly affected by the intermediate forms and the mixture of the forms reveals that the transformation to a more stable form may occur during storage.

Previous studies reported the use of NIR and Raman spectroscopy to quantify CBZF3 and CBZDH in binary mixtures (Rantanen, Wikström et al. 2005a; Tian, Zhang et al. 2007). The results from this thesis reveal that the quantification of quaternary mixtures of four CBZ solid-state forms can be performed using NIR and Raman spectroscopy (V). All solid-state forms that were present during the isothermal dehydration, identified with PLS-DA model, were also included in the quaternary calibration model. In the Raman spectroscopy model, the first three latent variables

differentiated between the solid-state forms, and the fourth latent variable represented the temperature effects on the spectra (**V**, Figs. 3b and c). In the NIR spectroscopy model all of the five latent variables used explained the variation in the spectra due to both the solid-state forms and temperature, and the model did not separate the two sources of variation into different latent variables.

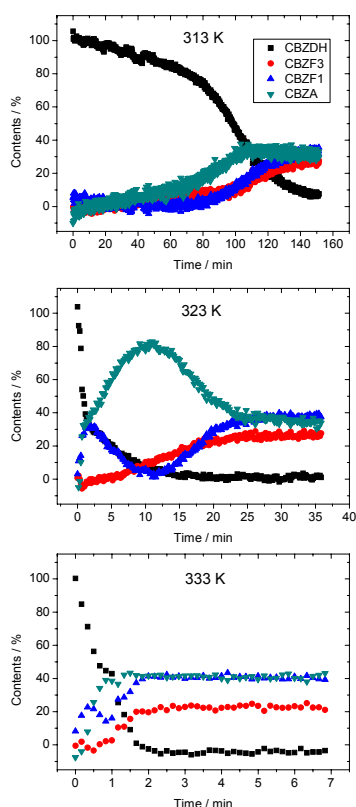


Figure 10. Quantification of CBZ solid-state forms during isothermal dehydration at 313, 323 and 333 K using Raman spectroscopy.

Although, NIR spectroscopy could quantitatively monitor the dehydration of CBZDH, it was unable to differentiate between the anhydrous forms of CBZ during and after the dehydration, due to subtle differences between the spectra of the forms and the low resolution of NIR spectroscopy. Thus, the method could not be used for quantification of the anhydrous forms during dehydration. However, Raman spectroscopy could monitor and quantify the multiple solid-state forms of CBZ during isothermal dehydration *in situ* (Fig. 10).

The Raman spectroscopy PLS regression models revealed the effect of temperature on the dehydration in more detail (Fig. 10). The formation of intermediate forms CBZA and CBZF1 depended largely on the temperature. The final product of dehydration was a mixture of CBZF3, CBZF1 and CBZA at all investigated temperatures. More CBZA formed during the dehydration at 323 K. At 313 and 338 K the CBZA content gradually increased and reached the final level of 35% and 40%, respectively.

5.2.3 Theophylline (II, IV)

5.2.3.1 Qualitative investigation of dehydration from compacts using TPS (II)

The dehydration for TPMH between 308 and 343 K measured by DSC was in good agreement with literature (**IV**, Suzuki, Shimomura et al. 1989). Previously, the dehydration of TPMH has been investigated using different analysis techniques and under various humidity and

RESULTS AND DISCUSSION

temperature conditions (Duddu, Das et al. 1995; Räsänen, Rantanen et al. 2001; Ahlqvist and Taylor 2002; Suihko, Ketolainen et al. 1997; Vora, Buckton et al. 2004). For TPMH, the studies performed at various environmental conditions are important, because similarly to CBZDH, the dehydration is considerably affected by temperature and humidity (Duddu, Das et al. 1995; Ledwidge and Corrigan 1997; Amado, Nolasco et al. 2007). In addition to different behaviour during dehydration, the anhydrous form prepared by heating at different dehydration temperatures has different moisture absorption and dissolution characteristics (Ono, Tozuka et al. 2001). To obtain additional information about the dehydration at the molecular level and investigate the dehydration behaviour from compacts, it was of interest to monitor the dehydration of TPMH using *in situ* TPS.

Although the dehydration of TPMH has been found to occur via a metastable form (Phadnis and Suryanarayanan 1997; Suihko, Ketolainen et al. 1997; Amado, Nolasco et al. 2007), the results from TPS do not corroborate this behaviour. Instead the formation of stable anhydrous TP was detected. The anhydrate spectrum matched with recently reported TPS spectra of form II reported by Upadhya et al. (Upadhya, Nguyen et al. 2006). It is possible that the concentration of the metastable form was too low to be detected by TPS. However, because the dehydration was performed using compacts, the dehydration conditions were different. The polymer matrix shielded the TPMH inside the mixed compacts, and substantially inhibited the dehydration. The water had to diffuse out of the compact, and then the dehydration from the compact may occur. According to the literature, the dehydration occurs as one step process in closed conditions in DSC without the formation of metastable phase (Suihko, Ketolainen et al. 1997). In open conditions a two step process has been proposed (Suihko, Ketolainen et al. 1997).

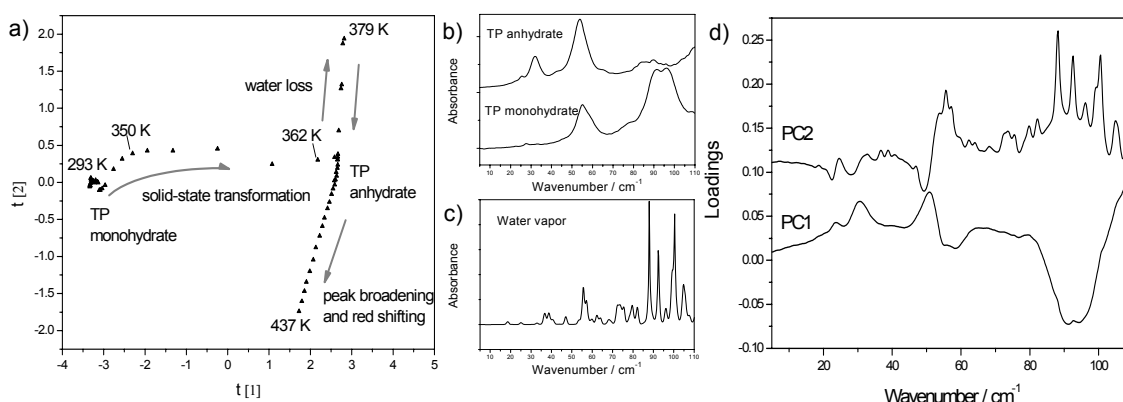


Figure 11. Monitoring non-isothermal dehydration by TPS. a) PC1 versus PC2 scores plot, b) TP anhydrate and TPMH spectra, c) water vapour spectrum, and d) PC1 and PC2 loadings plot.

Although, the information about dehydration is available and included in spectral data, it is visually difficult to observe and detect the changes in spectra. Therefore PCA was performed to interpret the dehydration data further and help to visualise the process (*Fig. 11*). The loadings of PC1 showed the spectral features of TPMH and TP anhydrate forms (*Fig. 11b*) and loadings of PC2 revealed the characteristic peaks from water vapour spectrum (*Fig. 11c*). Therefore, from the scores and loading plots it is seen that PC1 captured the solid-state transformation and PC2 monitored the water vapour leaving the sample and the effect of temperature on the TPS spectra (*Fig. 11*). In addition to the solid-state information, the molecular rotations of water in gaseous phase are easily detected by TPS. When the dehydration of TPMH from compacts was monitored using TPS in non-isothermal heating mode the characteristic water vapour peaks were identified from dehydration spectra. Furthermore, with PCA, it was possible to detect the onset and end-set temperatures for dehydration, and determine the specific time for water loss.

5.3 *In-line process monitoring during fluidised bed drying (IV, V)*

TPMH and CBZDH granules were heated at variable isothermal temperatures in a miniaturised fluidised bed dryer during which the dehydration of the granules occurred. This was confirmed by XRPD and Karl Fischer titration. The process was monitored using in-line NIR and Raman spectroscopy for TP (**IV**), and Raman spectroscopy for CBZ (**V**).

5.3.1 **Theophylline (IV)**

In contrast to the results of *in situ* TPS dehydration from compacts the dehydration from TPMH (Sun, Zhou et al. 2002) to anhydrous TP form II (Ebisuzaki, 1997) occurred via a metastable form in a fluidised bed dryer. This behaviour has previously been reported also during off-line monitoring of TP dehydration in the fluidised bed dryer (Airaksinen, Karjalainen et al. 2004). The dehydration from granules was successfully monitored with NIR and Raman spectroscopy together with PLS regression. Both methods showed the end-point of dehydration and the amount of hydrate was monitored in-line during heating. At 333 K, faster dehydration was detected compared to at 328 K (**IV**, Fig.7).

From the dehydration profiles, with NIR spectroscopy, it appears that complete dehydration occurs, but with Raman spectroscopy only a slight downward trend in the amount of TPMH is

observed (**IV**, Fig.7). These observations prove that Raman spectroscopy PLS models are affected by the presence of some other form. In this case, it was confirmed by XRPD, that the granules after the fluidisation experiment were anhydrous, but there was a metastable form present. Differences seen in the PLS model predictions for NIR and Raman spectroscopy, can be correlated to the previously explained different sensitivities of the vibrational spectroscopic techniques. NIR spectroscopy models mainly represent the amount of water during dehydration and Raman reveals the structural information about the transformation. Both NIR and Raman spectroscopy revealed complementary information about the overall process. However, because the PLS models did not include any other forms than TPMH and the stable anhydrate, the models were incapable of predicting the exact amount of the metastable form.

5.3.2 Carbamazepine (**V**)

Fluidised bed drying at 323 K monitored with in-line Raman spectroscopy revealed similar behaviour compared with dehydration on hot-stage. The only differences were related with the kinetics of the transformation. This is likely to be due to the different conditions and sample parameters (granules versus powder; fluidisation chamber versus hot-stage, moving versus static sample). The dehydration at 323 K was finished after approximately 45 min, and other solid-state changes were finished after 1 h (**V**, Fig.7). Therefore the process was slower in the fluidised bed dryer compared with hot-stage measurements where the dehydration was finished after 17 min. Although, the microscale fluidised bed mimics the real process environment, the parameters are not exactly the same and the results have to be evaluated carefully. For instance, the granule size used in the fluidised bed chamber was much larger than in real life process environment after granulation, therefore the dehydration possibility and the rate of dehydration might be different.

Multiple solid-state forms occurring during the dehydration of CBZDH were detected and quantified by Raman spectroscopy. In this case, the PLS regression models included all the solid-state forms that were known to appear during dehydration, and therefore, the quantification of all the forms was performed. It was seen that during the dehydration at 323 K, the dehydration went through CBZA (approximately 60% amorphous) to the mixture of anhydrous CBZF3, CBZF1, and CBZA (**V**, Fig. 7).

5.4 ***Increasing the understanding with multivariate modelling (I - V)***

Modelling of dehydration helped to predict the behaviour of pharmaceutical hydrates and increase the understanding of the solid-state changes at the molecular level. *In situ* spectral data and model predictions revealed the dehydration behaviour of these model drugs and allowed identification of the solid-state forms during and after water removal. Accordingly, this thesis showed that it is important to determine the multiple solid-state forms which may occur during dehydration prior to quantification. In the model construction phase, all possible solid-state forms need to be considered. This knowledge was further used to develop the model for quantification and in-line process monitoring - during the fluidised bed drying. The intermediate forms (metastable or amorphous) or mixture of the forms can only be quantified during dehydration if all these forms are known and included during the model development stage.

Vibrational spectroscopy techniques have characteristics needed for real time process monitoring and consequently for monitoring and controlling the process directly in-line. Deeper insight into the dehydration behaviour at the molecular level such as that obtained in this study is needed for process control. The quality and performance of the API in a solid dosage form can be guaranteed only if the properties of an API are known under different processing steps and environmental conditions. The use of complementary techniques offers a good approach that can be used to increase the overall process and product understanding. However, not all techniques should always be used simultaneously. The choice of the technique(s) depends on the parameters that need to be monitored and controlled within the unit operation during manufacturing. Although, the approach developed in this thesis is not yet a feed-back control method, it provides the basis for feed-back control. The overall control of the process can be achieved as a next step when the process parameters and conditions are known and understood.

6 SUMMARY AND CONCLUSIONS

The results presented in this thesis revealed that vibrational spectroscopy (NIR, Raman, and TPS) and multivariate modelling can be used to monitor and investigate dehydration behaviour *in situ* and in-line during the unit operation, fluidised bed drying.

All three spectroscopic methods proved complementary in the study of dehydration. The speed of spectroscopic methods makes them more suitable for monitoring dehydration than XRPD and vibrational spectroscopy directly gives solid-state structure information, unlike TGA. TPS detects the intermolecular phonon modes and Raman spectroscopy detects mostly the intramolecular vibrations. Both techniques revealed information about the crystal structure changes. Furthermore, the information about the molecular rotations of water in gaseous phase was obtained from TPS measurements. NIR spectroscopy, on the other hand was more sensitive to water content and the hydrogen bonding environment of water molecules.

The following specific conclusions can be drawn from this study:

- NIR, Raman, and TPS spectra distinguished between the anhydrous and hydrate forms of model drugs, and allowed monitoring of the solid-state transformations.
- PCA was found to be an excellent tool to interpret the spectral changes by separating the different sources of spectral variation into different PCs. The first PC versus temperature plots and also score one versus score two plots allowed monitoring of the *in situ* dehydration behaviour. The dehydration can be investigated qualitatively by PLS-DA. The constructed PLS-DA models enabled the monitoring of multiple forms appearing during dehydration, which should be considered when developing in/on-line methods for process control purposes.
- For the first time multiple spectroscopic techniques were used to monitor solid-state dehydration in compacts. The sample preparation method affected the dehydration behaviour of PRXMH from compacts. TPS proved to be a suitable technique to monitor the phase transformation within the compacts. Raman and reflectance NIR spectroscopy measurements were limited by the small sampling volume and interference from the polymer which formed the matrix of the compacts. However, all three spectroscopic techniques allowed *in situ* monitoring of non-isothermal PRXMH dehydration from near the surface of the compacts. Together, TPS and NIR and Raman spectroscopy simultaneously provided information on solid-state transformations, free water within

and on the surface of the compact, and water vapour as it leaves the compact, thus increasing understanding of dehydration from within compacts.

- PLS regression was found to be a good technique to conduct quantitative analysis of the spectral data. When quantitative analysis is performed, all known forms should be included in the calibration model, since this is prerequisite for the quantification of all the solid-state forms. For the first time the quantification of four CBZ solid-state forms was performed using NIR and Raman spectroscopy. Raman spectroscopy was found to have higher spectral resolution which allowed quantifying two PRX forms and all four CBZ solid-state forms during isothermal dehydration. NIR spectroscopy, on the other hand, revealed complementary information about the dehydration from PRXMH to PRXAH, showing good agreement with Raman spectroscopy and TGA results. However, NIR spectroscopy was incapable of differentiating between the anhydrous solid-state forms of CBZ during dehydration.
- The solid-state transformations were monitored successfully during fluidised bed drying. For TPMH, both NIR and Raman spectroscopy allowed in-line monitoring of dehydration. For CBZDH only Raman spectroscopy could be used for monitoring the multiple solid-state transformations.

Although the model substances all have anhydrous polymorphic forms, only the channel hydrates TPMH and CBZDH showed complicated dehydration behaviour due to polymorphism and the presence of intermediate phases. The presence of metastable TP and lower crystallinity phase of CBZ were detected during dehydration. Thus, depending on the crystal structure of the hydrate and the water molecule arrangement the dehydration mechanisms varied largely. The dehydration kinetics, and in case of TPMH and CBZDH, also the solid-state forms, were largely affected by the temperature. Due to the presence of strong PRX-water hydrogen bonding network in the structure PRX had higher dehydration temperature ranges. The dehydration showed a direct transformation from PRXMH to PRXAH. The changes in structure and conformation occurred simultaneously with water loss.

This thesis provides a basis for monitoring solid-state changes in both pure pharmaceutical materials and solid dosage forms using NIR, and Raman spectroscopy and TPS. The developed approach is proposed for improved process monitoring and understanding during pharmaceutical manufacturing.

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