Division of Pharmaceutical Technology
Faculty of Pharmacy
University of Helsinki
Finland

Increasing process understanding of wet granulation by spectroscopic methods and dimension reduction tools

by

Anna Cecilia Jørgensen

Academic dissertation

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Supervisors: Docent Jukka Rantanen
Viikki Drug Discovery Technology Center
Division of Pharmaceutical Technology
Faculty of Pharmacy
University of Helsinki
Finland

Prof. Jouko Yliruusi
Division of Pharmaceutical Technology
Faculty of Pharmacy
University of Helsinki
Finland

Reviewers: Prof. Jari Yli-Kauhaluoma
Division of Pharmaceutical Chemistry
Faculty of Pharmacy
University of Helsinki
Finland

Dr. Poul Bertelsen
Nycomed Danmark ApS
Roskilde
Denmark

Opponent: Prof. Kenneth R. Morris
Department of Industrial and Physical Pharmacy
Purdue University
West Lafayette
Indiana
United States of America

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ABSTRACT

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Wet granulation is a common unit operation applied in the pharmaceutical industry. It is a complex process where several interrelated phenomena take place simultaneously. Moreover, it exposes the processed materials to harsh conditions which may alter the solid-state of these. Thus, in order to increase process understanding of wet granulation, methods providing real-time information from the process would be valuable.

The aim of the present study was to investigate the use of spectroscopic methods, near-infrared (NIR) and Raman spectroscopy, in elucidating phenomena taking place during wet granulation. More specifically, a processing-induced transformation, hydrate formation, which takes place during wet granulation, was studied. In addition, the use of near-infrared spectroscopy in the process monitoring of high-shear wet granulation was studied by comparing it to impeller torque measurements, which is an established process monitoring method. The measurements were performed off- or at-line. Moreover, the difficulty to grasp the large data amounts produced by different process monitoring methods was addressed by combining the data and visualising it with projection methods. Two different approaches were investigated, principal components analysis and self-organizing maps, which are linear and non-linear methods, respectively.

It was possible to follow the processing-induced transformation by both spectroscopic methods. Common excipients did not disable the measurements, but altered the rate of transformation. NIR reflected also macroscopic changes taking place during the high-shear granulation process, such as the increase in size and consolidation of the agglomerates. The combination of process data enabled the study of the state of the process in a way which none of the individual process measurements allowed. Both projection methods were able to solve the task of visualising the state of the process. Hence, the use of all of the available process data in a multidisciplinary way, allowed by the projection methods, may contribute to the creation of better process understanding.

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PREFACE

This study was carried out at the Division of Pharmaceutical Technology, Faculty of Pharmacy, University of Helsinki, Finland, during the years 2000-2004. The experimental work was performed at the above mentioned institution; at the Department of Pharmaceutics at the Danish University of Pharmaceutical Sciences, Copenhagen, Denmark; and at Product Development, AstraZeneca R&D Mölndal, Sweden.

This thesis is based on original publications listed on page v. Some readers may find it useful to read those before reading the experimental and results & discussion sections of the thesis. The terms granule and agglomerate are used interchangeably in this thesis, although it is recognized that the definition of agglomerate is usually broader.

List of abbreviations and acronyms

API	Active pharmaceutical ingredient
DSC	Differential scanning calorimetry
EMEA	European Agency for the Evaluation of Medicinal Products
FDA	Food and Drug Administration (in the United States)
ICH	International Conference on Harmonization
IR	Infrared
MCC	Microcrystalline cellulose
MTR	Mixer torque rheometry *or* mixer torque rheometer
NIR	Near-infrared
NMR	Nuclear magnetic resonance
PAC	Process analytical chemistry
PAT	Process analytical technology
PCA	Principal components analysis *or* principal component analysis
Ph.Eur.	European Pharmacopoeia
PITs	Processing-induced transformations
PLS	Partial least squares *or* projection to latent structures
SMCC	Silicified microcrystalline cellulose
SOM	Self-organizing map
T_f	Temperature of fusion, melting temperature
T_t	Transition temperature
TG	Thermogravimetry
USP	United States Pharmacopeia
XRPD	X-ray powder diffraction

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-VI.

I Räsänen, E., Rantanen, J., Jørgensen, A., Karjalainen, M., Paakkari, T. and Yliruusi, J., 2001. Novel identification of pseudopolymorfic changes of theophylline during wet granulation using near infrared spectroscopy. *Journal of Pharmaceutical Sciences* **90** 389-396.

II Jørgensen, A., Rantanen, J., Karjalainen, M., Khriachtchev, L., Räsänen, E. and Yliruusi, J., 2002. Hydrate formation during wet granulation studied by spectroscopic methods and multivariate analysis. *Pharmaceutical Research* **19** 1285-1291.

III Airaksinen, S., Luukkonen, P., Jørgensen, A., Karjalainen, M., Rantanen, J. and Yliruusi, J., 2003. Effects of excipients on hydrate formation in wet masses containing theophylline. *Journal of Pharmaceutical Sciences* **92** 516-528.

IV Jørgensen, A.C., Airaksinen, S., Karjalainen, M., Luukkonen, P., Rantanen, J. and Yliruusi, J., 2004. Role of excipients in hydrate formation kinetics of theophylline in wet masses studied by near-infrared spectroscopy. *European Journal of Pharmaceutical Sciences* (accepted).

V Jørgensen, A.C., Luukkonen, P., Rantanen, J., Schæfer, T., Juppo, A.M. and Yliruusi, J., 2004. Comparison of torque measurements and near-infrared spectroscopy in characterization of a wet granulation process. *Journal of Pharmaceutical Sciences* **93** 2232-2243.

VI Jørgensen, A.C., Rantanen, J., Luukkonen, P., Laine, S. and Yliruusi, J., 2004. Visualization of a pharmaceutical unit operation: wet granulation. *Analytical Chemistry* (accepted).

1 INTRODUCTION

The ability to deliver a consistent quality is a prerequisite for remaining in business in an industry as highly regulated and quality critical as the pharmaceutical sector (Miller, 2003). However, it is not easy to achieve the quality required. It has been estimated that 5-10% of pharmaceutical product batches have to be reworked or discarded, because they do not meet their specifications (Abboud and Hensley, 2003). Additionally, in the research based pharmaceutical field, the top 16 companies spent in 2001 36% of their costs on manufacturing, whereas the research and development expenses were below the half of this (16%). Thus, there is an increasing focus on making the manufacturing more effective and optimizing processes to deliver consistent quality. In order to achieve this, the level of process understanding has to be increased. The pharmaceutical industry has been hesitant to implement new methods for process analysis and quality control due to strict regulatory requirements. The picture has now changed, because the U.S. regulatory agency encourages in implementing new technologies (FDA, 2003a,b).

Wet granulation is a commonly used unit operation in the pharmaceutical industry. Granulation is mainly performed to produce suitable feed material for tabletting or capsule filling. The objective of granulation is to improve powder flow and handling, decrease dustiness, and prevent segregation of the constituents of the product. Wet granulation is often carried out utilizing a high-shear mixer. The high-shear granulation process is a rapid process which is susceptible for over-wetting. Thus, the liquid amount added is critical and the optimal amount is affected by the properties of the raw materials. Some of the excipients used in pharmaceutical solid dosage forms are of natural origin and their properties vary in a way that has an impact on the granulation process (Parker and Rowe, 1991; Rowe and Sadeghnejad, 1987). Power consumption of the impeller motor and the impeller torque have been applied to monitor the rheological properties of the wet mass during agglomeration and, thereby, have been used to determine the end-point of water addition. However, these methods are affected by the equipment variables. Hence, additional process monitoring techniques would be valuable.

During the processing of pharmaceutical raw materials into drug products, the materials are often exposed to rather harsh conditions. This can lead to processing-

induced transformations (PITs), where the active pharmaceutical ingredient or the excipients undergo a phase transition or transitions during the process (York, 1983; Morris *et al.*, 2001). Wet granulation is an example of a process where processing-induced transformations may take place. Wet granulation, with the consecutive drying process, involves increased temperatures, a significant amount of moisture and mechanical stress, all being able to induce a phase transition. In order to state that a wet granulation process is understood and fully under control, one should also be able to monitor the processing-induced transformations. The problems encountered due to processing-induced transformations during wet agglomeration can be solved by replacing the wet agglomeration with dry agglomeration or by passing the agglomeration step using direct compression. Nevertheless, these processes have their own difficulties. All drugs are not suitable for dry agglomeration or direct compression due to poor compactibility, bad flow or segregation. Wet agglomeration is commonly used because it genuinely adds value in terms of flowability and compactibility, and it improves the drug homogeneity. It has even been reported that wet granulation has been used to deliberately induce a phase transformation in order to achieve better compactibility of chlorpromazine hydrochloride (Wong and Mitchell, 1992).

Hydrate formation is a PIT that may take place during wet granulation. The dissolution rates of hydrates and the anhydrous counterparts often differ (Shefter and Higuchi, 1963). Thus, a different hydration state may lead to a different dissolution profile of the active and may also potentially affect the bioavailability of the drug. Variations in dissolution due to hydrate formation has been reported, e.g. carbamazepine (Kahela *et al.*, 1983), theophylline (Herman *et al.*, 1989) and nitrofurantoin (Otsuka *et al.*, 1991). As noted above, wet granulation may cause hydrate formation. During the next step in processing, drying of the granules, the hydrate may transform into a metastable form, whose dissolution also differs from the dissolution of the stable anhydrous form, e.g. theophylline (Phadnis and Suryanarayanan, 1997). Hence, it would be valuable to monitor the solid-state of drugs during processing.

2 THEORY AND LITERATURE SURVEY

2.1 Solid-state of drugs

2.1.1 Definitions

Drug compounds can usually exist as crystalline or amorphous solids. A crystalline material has a defined three dimensional structure, so called crystal lattice, in which structural units (unit cells) are repeated in a regular manner. In the amorphous state, such order cannot be found, and therefore, these materials do not posses any distinguishable crystal lattice. However, some short-range order can be present. The different ways of producing of amorphous glasses may result in amorphous phases with distinctive properties (Hancock *et al.*, 2002). It has been suggested that these phases should be called pseudo-polyamorphs (Hancock *et al.*, 2002).

Many drug substances can exist in different crystal packing arrangements. This phenomenon is called polymorphism. Analogously, the different crystal forms are termed polymorphs. In some cases, the difference in crystal packing arises from different conformations of the molecules. Solvent molecules can be incorporated in the crystal lattice in either stoichiometric or nonstoichiometric proportions. These forms are called solvates or pseudopolymorphs. If the adduct is water, the form is termed a hydrate. The water molecule is, due to its small size and hydrogen bonding capacity, suited to fill voids in crystal structures and to bond organic molecules into stable structures (Byrn *et al.*, 1999). In addition, the hydrates are of special interest due to the abundance of water in the atmosphere and its widespread use in the final crystallization step and processing of pharmaceuticals.

Hydrates are sometimes classified structurally by dividing them into classes that are discernible by commonly available analytical techniques (Morris, 1999; Morris and Rodríguez-Hornedo, 1993). In *isolated site hydrates*, the water molecules are isolated from direct contact to other water molecules by drug molecules. The hydrates in this class are characterized by sharp differential scanning calorimetry (DSC) endotherms, and the dehydration product may be amorphous and unstable. In *channel hydrates*, the water molecules construct chains along an axis of the lattice. In some cases, these hydrates take up additional moisture when exposed to high relative humidity in non-

stoichometric proportions (*expanded channels*), or the water forms a two dimensional structure in the lattice (*planar hydrates*). The channel hydrates are often characterized by an early onset of dehydration. *Dehydrated hydrates* arise from dehydration of usually channel hydrates that leaves an intact anhydrous structure similar to the hydrated structure. Some consider dehydrated hydrates as a hydrate class, while others regard these as a polymorph. *Ion associated hydrates* contain metal ion coordinated water. Dehydration at high temperatures is distinctive for this class due to the relatively strong metal-water interaction.

2.1.2 Relative stability of the solid states

Only one polymorph can be stable under defined conditions of temperature and pressure (Grant, 1999). The difference in Gibbs free energy (G) acts as the driving force for a polymorphic transformation at constant temperature and pressure, and is given by

$$\Delta G = \Delta H - T\Delta S \tag{1}$$

where H is enthalpy, T is temperature and S is entropy. The total energy of a system is represented by the enthalpy at a constant pressure. The T·S term represents the energy of the system that is associated with the disorder of the molecules. The stable form has the lowest Gibbs free energy and, therefore, the lowest vapour pressure, thermodynamic activity and solubility. If an unstable phase transforms at a very low rate, it can be termed metastable. The relative stability of polymorphs can be described by concepts of *enantiotropy* and *monotropy*. For enantiotropes the relative thermodynamic stability is a function of temperature and pressure. Thus, a definitive transition temperature exists and the transition is reversible. The free energy curves of the polymorphs cross at the transition temperature (Fig. 1a). In a monotropic system, one polymorph is stable at all temperatures below the melting point and the other polymorph is unstable. In

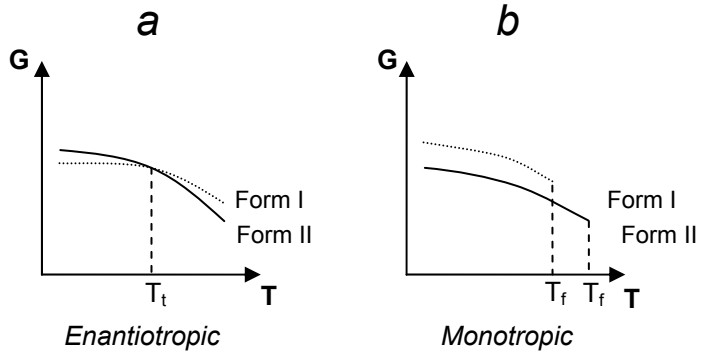

Fig. 1. Schematic graphs of Gibbs free energy (G) versus temperature for a) an enantiotropic and b) a monotropic system. The form having the lowest free energy is the most stable. Modified from Byrn et al. (1999) and Grant (1999).

this case, the free energy curves do not cross (Fig. 1b) and thus a reversible transition cannot be observed below the melting point.

How do then the unstable polymorphs come into being, if the energetics favours the most stable form? When a drug is crystallised, e.g. by cooling down a supersaturated solution, the form initially formed is not the one with the lowest free energy, but the one lying nearest in free energy to the original state. This empirical rule based on kinetics is known as the Ostwald's step rule (Grant, 1999). This rule is however not always obeyed. Moreover, the kinetics of different polymorphs is not governed solely by a reduction of free energy, but structural factors may play a part as well (Brittain and Byrn, 1999).

The amorphous state exhibits a higher molar enthalpy than the crystalline state due to the lack of stabilizing lattice energy (Grant, 1999; Hancock and Zografi, 1997). In addition, the molar entropy of the amorphous form exceeds that of the crystalline state, because there is no long-range order. Thus, the amorphous state may have some advantages such as higher solubility (Hancock and Parks, 2000) than the crystalline counterpart, but then again, the chemical and physical stability are lower than those of the crystalline state. The processing history of amorphous glasses may result in different kinetic properties (Hancock *et al.*, 2002).

When a water molecule is able to be incorporated in the crystal lattice, the relative stability is not solely governed by pressure and temperature, but also by the activity of water. The equilibrium between a hydrate and its anhydrate (Khankari and Grant, 1995; Morris, 1999) may be represented by the relationship

$$\mathrm{A(solid)} + m\mathrm{H_2O} \underset{}{\overset{K_h}{\rightleftharpoons}} \mathrm{A} \cdot m\mathrm{H_2O(solid)}\,, \tag{2}$$

$$K_h = \frac{a[\mathrm{A} \cdot m\mathrm{H_2O(solid)}]}{a[\mathrm{A(solid)}]a[\mathrm{H_2O}]^m} \tag{3}$$

where K_h is the equilibrium constant and $a[\mathrm{A} \cdot m\mathrm{H_2O(solid)}]$, $a[\mathrm{A(solid)}]$, $a[\mathrm{H_2O}]$ are the activities of the hydrate, anhydrate and water, respectively. The hydrate will be more stable than the anhydrate when $K_h > 1$, i.e. when

$$a[\mathrm{H_2O}] > \left(\frac{a[\mathrm{A} \cdot m\mathrm{H_2O(solid)}]}{a[\mathrm{A(solid)}K_\mathrm{h}]} \right)^{1/m} . \quad (4)$$

If the pure solids are taken as the standard states (i.e. the states with unit activity) for the hydrate and anhydrate, then Eq. (3) simplifies to give Eq. (5).

$$K_\mathrm{h} = a[\mathrm{H_2O}]^{-m} \quad (5)$$

Thus, the stability of a hydrate relative to the anhydrate, or a higher hydrate relative to a lower hydrate, depends on the activity of water in the surrounding medium, e.g. the vapour phase or the crystallization medium. In addition to this basic rule, the amount of crystal defects affects the stability of hydrates (Byrn and Lin, 1976; Irwin and Iqbal, 1991; Kitamura *et al.*, 1989; Otsuka and Kaneniwa, 1984).

The model drugs applied in this thesis, theophylline and caffeine, have related structures, caffeine having an additional methyl group compared to theophylline (Fig 2). Theophylline is known to exist in two enantiotropically related anhydrous forms, I and II where the form II is stable at room temperature (Suzuki *et al.*, 1989). In addition, a metastable form that is monotropically related to form II has been reported (Phadnis and Suryanarayanan, 1997). Moreover, theophylline exists as a monohydrate in elevated humidities (Otsuka *et al.*, 1990) or aqueous solutions (Bogardus, 1983). The hydrate has been reported to be the stable form at a water activity above 0.25 (25 °C) (Zhu *et al.*, 1996) or 0.64 (30 °C) (Ticehurst *et al.*, 2002). Anhydrous caffeine exists as low and high temperature forms (Suzuki *et al.*, 1985). Moreover, caffeine has been reported to exist as a 4/5-hydrate (Bothe and Cammenga, 1980; Sutor, 1958b). The 4/5-hydrate is the stable form at relative humidities above approx. 75% (Griesser and Burger, 1995). The hydrates of theophylline and caffeine are channel hydrates (Sun *et al.*, 2002; Sutor, 1958a,b), and their dehydration starts at the ends of the water tunnels of the crystals with a threshold temperature of 47 and 44 °C, respectively (Byrn and Lin, 1976; Perrier and Byrn, 1982).

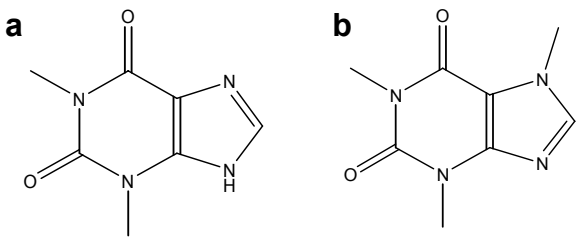

Fig. 2. Structural formulas of a) theophylline and b) caffeine.

2.1.3 Impact of different solid states on the properties of solids

Differences in molecular packing affect the physical properties of a solid due to difference in the dimensions, shape, symmetry, number of molecules, and void volumes of the unit cells of the polymorphs or solvates (Table 1). Differences in energetics of intermolecular interactions give rise to differences in thermodynamic properties (Table 1, Fig. 3). Additionally, the polymorphs may differ in kinetic, surface and mechanical properties (Table 1). Moreover, differences can arise in interaction with electromagnetic radiation leading to differences in spectroscopic properties.

Table 1. Physical properties that may differ between polymorphs (or solvates) of the same compound.[a]

Physical properties	**Examples**
Packing	Molar volume and density, refractive index, conductivity, hygroscopicity
Thermodynamic	Melting and sublimation temperature, enthalpy, heat capacity, free energy, vapour pressure, solubility
Spectroscopic	Vibrational transitions (IR, Raman), rotational transitions (Far-IR, microwave), nuclear spin transitions (NMR)
Kinetic	Dissolution rate, rates of solid-state reactions, stability
Surface	Surface free energy, interfacial tensions, crystal habit
Mechanical	Hardness, tensile strength, compactibility, tabletting, handling, flow

[a] Modified from Clas (2003) and Grant (1999).

The pharmaceutical relevance of the differences varies from system to system. These differences potentially affect many aspects of pharmaceutical product development, such as processing and stability (Haleblian and McCrone, 1969), to name a few. If the difference in solubility is significant and the absorption of the drug is dissolution controlled, i.e. class II drug in the biopharmaceutics classification system (Amidon *et al.*, 1995), the polymorphism or solvate formation may have an impact on bioavailability. Although this chapter has concentrated on active drug substances, it should be noted that the polymorphism and pseudopolymorphism of excipients may affect the processing and performance of pharmaceuticals as well (Giron, 1990; York, 1983).

It is paramount to perform throughout solid-state characterization programs in early stages of drug development allowing the selection of the best form to market and to thereby prevent unpleasant surprises later on. An unfortunate example of the

difficulties encountered during characterization, is the case of ritonavir, where emergence of a new, less soluble polymorph led to the withdrawal of a formulation from the market (Bauer *et al.*, 2001; Clas, 2003). Some characterization approaches are presented in literature (Byrn *et al.*, 1995; Yu *et al.*, 1998). The regulatory authorities have also recognized the importance of polymorphism and require polymorphism screening for new chemical drug substances (ICH, 1999).

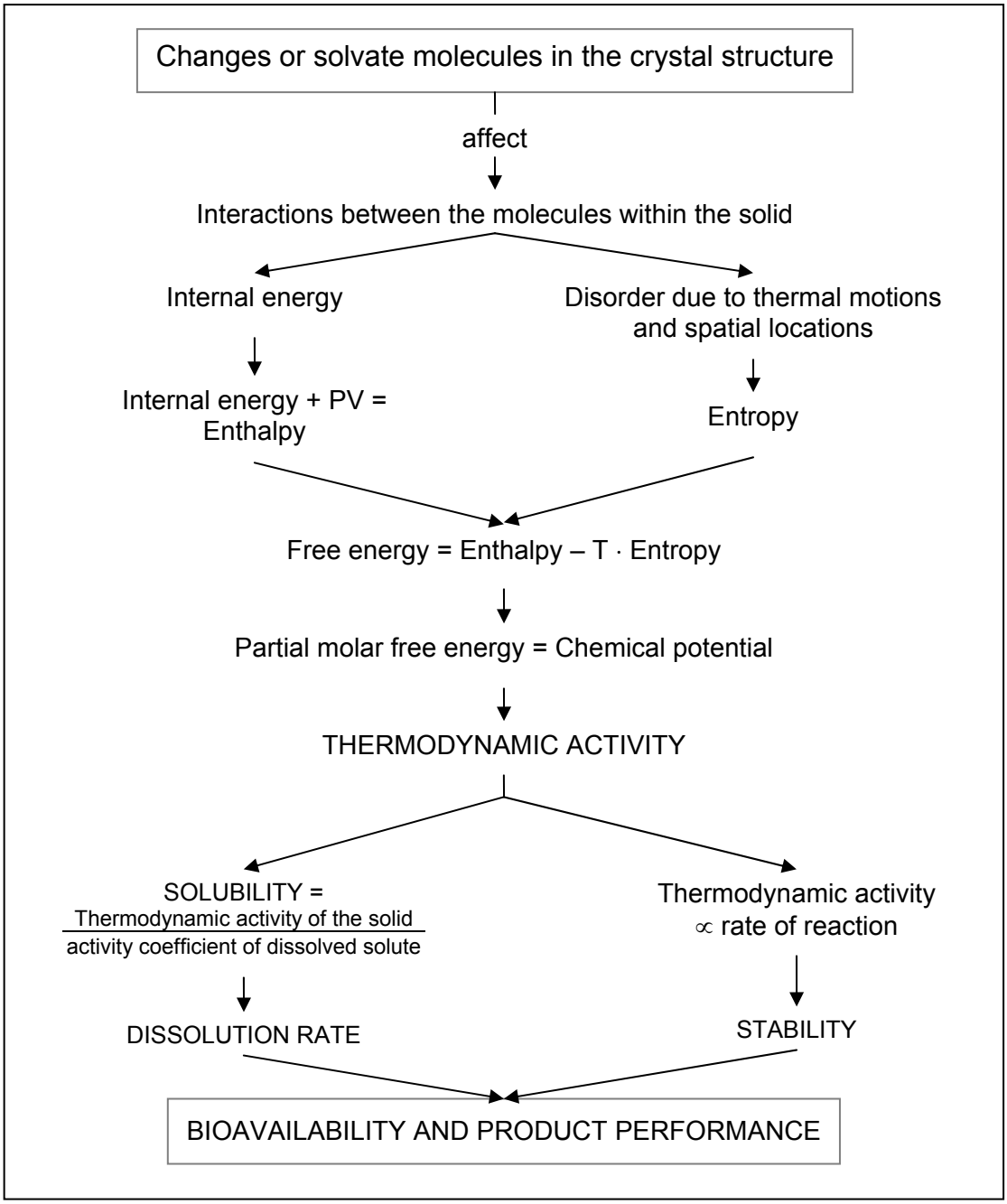

Fig. 3. Effect of polymorph conversion or solvate formation on the thermodynamic properties of a drug. Modified from Khankari and Grant (1995).

2.2 Techniques for analysing the solid state of drugs

In this chapter, the most common methods for characterization of the solid state are overviewed. In general, for effective characterization, the different characterization techniques have to be used in a multi-approach fashion combining results obtained by different methods.

2.2.1 X-ray diffraction

Single crystal x-ray diffraction is the ultimate technique for solving crystal structures. Diffraction takes place when radiation encounters a set of regularly spaced scattering objects, provided that the wavelength of the radiation is of the same order as the distance between the scattering centres (Brittain, 1999; Cullity and Stock, 2001). This is the case with x-rays and the periodic order of atoms in crystals, which have the wavelength and distance of 1-2 Å, respectively. Thus, x-ray diffraction can be used to study the structure of crystalline materials. The x-ray diffraction techniques are based on Bragg's law, which describes the diffraction of monochromatic x-ray radiation impinging on a plane of atoms. The parallel incident rays striking the plane at an angle θ are diffracted at the same angle. This reinforcement of the x-rays takes place when the distance between the molecular planes (*d*) is equal to a whole number of wavelengths (λ). The scattering angles can be therefore related to the spacings between planes of molecules in the lattice using Bragg's law:

$$n\lambda = 2d \sin\theta \tag{6}$$

where *n* is the order of the diffraction pattern, λ is the wavelength of the incident beam, *d* is the distance between the planes in the crystal and θ is the angle of beam diffraction.

In a fine powder, the different crystal faces are oriented randomly in all possible directions at the powder interface. This provides the basis for x-ray *powder* diffraction (XRPD), as the diffraction of this surface provides information on all possible atomic spacings in the crystal lattice. A single atom scatters an incident beam in all directions, and it is the structured crystal lattice that allows the diffraction only in a few directions. Therefore, if the structure lacks as in the amorphous state, scattering at all angles is detected.

XRPD is commonly used in identification and quantification of polymorphs (Stephenson *et al.*, 2001). It can also be used to quantify the amorphous content. With specialized techniques, high-quality powder diffraction patterns can also be used for solving crystal structures. XRPD is usually applied off-line, although a pharmaceutical in-line application has been published (Davis *et al.*, 2003). A disadvantage of x-ray diffraction in in-line use is the hazardous nature of the radiation.

2.2.2 Infrared spectroscopy

Infrared (IR) spectroscopy is based on absorption of light at a particular frequency by vibrating covalent bonds (Griffiths, 2002; Osborne *et al.*, 1993). The light has to have the same energy (frequency) as the molecular vibration to be absorbed. Only vibrations that result in changes in the dipole moment of a molecule can cause absorption in the infrared region. The fundamental frequency (v_0) of an atom-to-atom bond can be estimated from the vibration of a diatomic harmonic oscillator using Hooke's Law:

$$v_0 = \frac{1}{2\pi}\sqrt{\frac{k}{\mu}} \tag{7}$$

where k is the force constant, i.e. the relative strength of the bond, and μ is the reduced mass ($\mu=(m_1m_2)/(m_1+m_2)$). However, molecules cannot take up energy continuously. By solving a quantum mechanical wave equation for a simple harmonic oscillator, the possible energy levels can be calculated by

$$E_v = (v+\tfrac{1}{2})hv_0 \tag{8}$$

where v is the vibrational quantum number (0, 1, 2…) and h is the Planck's constant. The vibrational energies are quantized, and the promotion to the first exited state ($v=1$) requires the energy ΔE:

$$\Delta E = hv_0 = \frac{h}{2\pi}\sqrt{\frac{k}{\mu}}\,. \tag{9}$$

This model explains the fundamental absorption bands ($\Delta v=\pm 1$) which are observed in the middle infrared region (4000-200 cm^{-1}). Because the vibrational motion in different packing or conformational arrangements is potentially different, IR spectroscopy can be utilized for polymorphic investigations (Threlfall, 2002). It is the most common

spectroscopic technique in the analysis of solid state. Earlier, the sample preparation to alkali halide (KBr, KCl) pellets has compromised the solid-state characterization but this can be avoided by using the diffuse reflectance technique (Bugay, 2001).

2.2.3 Near-infrared spectroscopy

Absorbance in the near-infrared region (700-2500 nm or 14300-4000 cm^{-1}) originates from overtones ($\Delta v = \pm 2, \pm 3, \ldots$) and combinations of fundamental vibrations observed in the middle infrared region (Osborne *et al.*, 1993). Real molecules do not obey exactly the laws of simple harmonic oscillator, Eq. (8), and the Hooke's law, Eq. (7), due to coulombic repulsion between two nuclei in one end of the vibration and dissociation in the other extreme.

Vibration can be described as either stretching or bending. A change in the interatomic distance is called stretching and a change in the bond angle is called bending. The intensities of overtone and combination bands depend on the degree of anharmonicity. The stretching vibrations of bonds involving hydrogen have large amplitude, and therefore, this motion deviates most from harmonic. Thus, the majority of absorption bands observed in the near-infrared originate from stretching vibrations of XH groups, X being O, C, N or S, or combinations involving stretching and bending of these groups.

NIR spectroscopy is often conducted in reflectance mode which allows the measurement of solid samples (Osborne *et al.*, 1993). Reflectance spectroscopy measures the light reflected by the sample surface. This can be divided to specular and diffuse reflectance. The diffuse reflectance component contains the chemical information and is the component mainly employed in NIR spectroscopy. Many workers apply a relationship similar to the Beer-Lambert's law:

$$A = \log \frac{1}{R} \tag{10}$$

where A is apparent absorbance and R is the reflectance relative to a non-absorbing standard. The concentration is expected to be relative to the apparent absorbance ($c \propto \log 1/R$).

Like in mid-IR, the absorption bands in NIR spectra are sensitive to changes in hydrogen bonding and packing in the crystal lattice. Due to this NIR can be applied to analysis of the solid state. It has been used in identification of the desired polymorph (Aldridge *et al.*, 1996), polymorph quantitation (Luner *et al.*, 2000; Patel *et al.*, 2000) and the determination of crystallinity (Hogan and Buckton, 2001). In addition, hydrate water bands are sharper than other water bands, because the energic distribution of the OH vibrations is rather uniform, when the water molecules are bound into the crystal lattice. Bulk water has NIR absorption bands at around 760, 970, 1190, 1450 and 1940 nm (Curcio and Petty, 1951) having increasing absorptivity with increasing wavelength. The bands at around 760, 970 and 1450 nm are the third, second and first overtone, respectively. The 1190 nm band has been assigned to be a combination of symmetric stretching, bending and asymmetric stretching (Buijs and Choppin, 1963). Further, the 1940 nm band has been assigned to a combination of bending and asymmetric stretching (Choppin and Downey, 1972). The bands shift towards higher wavelength with increasing hydrogen bonding (Buijs and Choppin, 1963; Choppin and Violante, 1972; Fornés and Chaussidon, 1978; Iwamoto *et al.*, 1987; Maeda *et al.*, 1995). Although NIR is a rather new method compared to mid-IR, it is beginning to be an established technique especially in identification of raw materials. The method has been recently adopted to pharmacopoeias (Ph.Eur., 2004a; USP 27, 2003).

An advantage of NIR spectroscopy is that it can be applied in reflectance mode enabling non-invasive measurements due to the low molar absorptivities in the NIR region. Sample preparation in the traditional sense is not necessary, as spectra can be measured directly from solid materials. Moreover, glass is relatively transparent for NIR radiation. The measurements are non-destructive and fast. Systems performing 12 000 measurements per minute have been reported (Herkert *et al.*, 2001). However, the line widths of NIR are broad resulting in overlapping bands and making assignment of the different features of the spectra difficult. Moreover, the spectra are strongly influenced by factors that affect the path length of light propagating in the sample such as particle size and packing density of the sample. This phenomenon can, on the other hand, be considered as an advantage of NIR because it can be used to gather physical information (Dreassi *et al.*, 1995; Frake *et al.*, 1998; Ilari *et al.*, 1988; Kirsch and Drennen, 1999; Morisseau and Rhodes, 1997; O'Neil *et al.*, 1998).

2.2.4 Raman spectroscopy

Raman spectroscopy is based on measurement of frequency shifts of scattered light (Ferraro and Nakamoto, 1994; Ph.Eur., 2004b). If a sample is irradiated with monochromatic electromagnetic radiation (v_0), major part of the radiation is scattered elastically (Rayleigh scattering) and the frequency of the scattered light is the same as that of the incident beam (Fig. 4). However, a small fraction of the radiation is scattered inelastically (Raman scattering) with a frequency smaller (Stokes lines) or greater (anti-Stokes lines) than that of the incident beam ($v_0 \pm v_m$). These differences in the frequency are called Raman shifts. The Raman bands which are at a lower frequency as the incident beam, i.e. the Stokes lines, are often used alone due to their higher intensity at room temperature. As noted earlier, IR absorption requires a change in the dipole moment during the vibration. In Raman, a change in polarizability is necessary instead. In a qualitative sense, asymmetric vibrational modes and vibrations due to polar groups usually show strong IR absorption, while symmetric vibrational modes are typically strong Raman scatterers. Thus, Raman spectroscopy is often described as a complementary technique to IR.

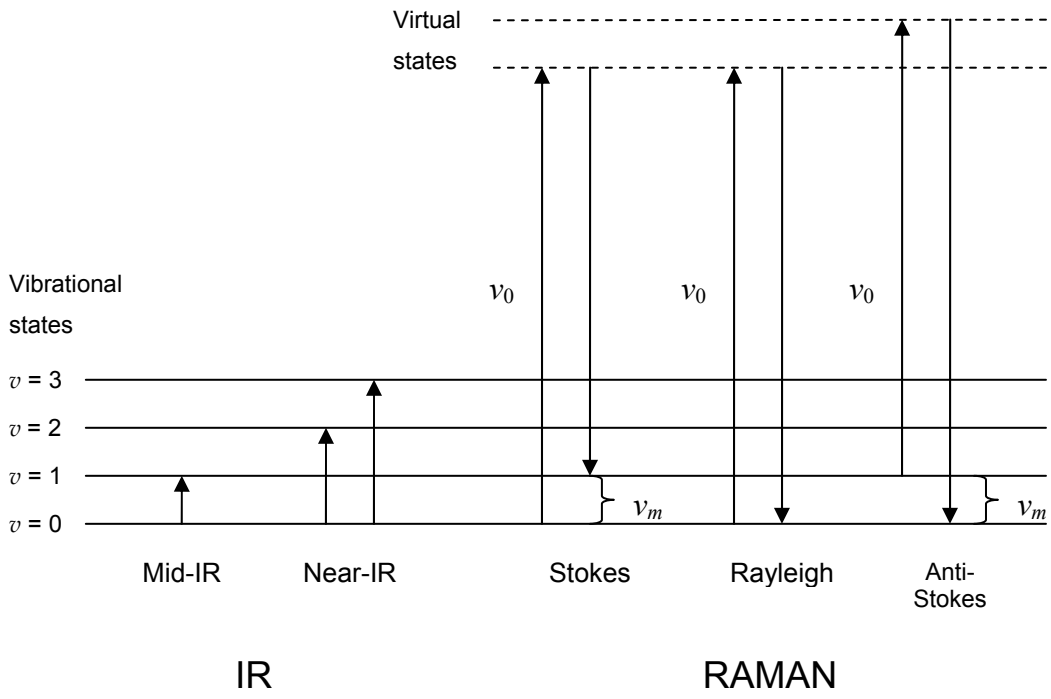

Fig. 4. Energy states involved in infrared and Raman spectroscopies. Modified from Lin-Vien et al. (1991) and Ferraro and Nakamoto (1994).

Raman spectroscopy has been utilized for distinguishing solid-state forms of drugs in bulk drug (Bolton and Prasad, 1981; Neville *et al.*, 1992; Szelagiewicz *et al.*, 1999), in slurries (Anquetil *et al.*, 2002; Starbuck *et al.*, 2002) during crystallization (Wang *et al.*, 2001) and in tablets (Taylor and Langkilde, 2000). In addition, it has been used for quantitation of polymorphs (Langkilde *et al.*, 1997; Pratiwi *et al.*, 2002) and crystallinity (Taylor and Zografi, 1998). Moreover, it has been utilized for granulation process development in order to find the process conditions that do not cause dissociation of a hydrochloride salt drug to its base (Williams *et al.*, 2004).

The major advantages (Vankeirsbilck *et al.*, 2002) of Raman spectroscopy are that measurements can be performed fast, directly from powders and spectra can be obtained through plastics and glass, i.e. products can be measured directly in their packages. Raman spectrometers can be coupled with fibre optic probing which enables on-line and non-invasive measurements. Water is a weak Raman scatter, and therefore, Raman can be applied for analysis of aqueous suspensions. In contrast to NIR, the Raman bands are well resolved. Some of the Raman instruments enable measurement of lattice vibrations (Bugay, 2001), so called phonons, which occur at low frequencies (400-50 cm^{-1}).

The lasers applied in Raman spectroscopy present some advantages and disadvantages. On one hand, the area excited by the lasers is small (Williams, 2001), so Raman measurements can be performed on relatively small sample amounts or can be used to study small particles in a matrix. On the other hand, the small area studied can lead to unrepresentative data, if the samples are inhomogeneous and care is not taken. The particle size of the samples affect the Raman spectra in some extent, but contradictory reports have been published on the direction of the effect (Pellow-Jarman *et al.*, 1996). Fluorescence, which overlays the Raman bands, is a problem connected to the use of lasers operating at the visible region. This can be in most cases avoided by using lasers in the NIR region. In addition, the samples may decompose thermally, if high excitation intensities are employed.

2.2.5 Nuclear magnetic resonance spectroscopy

Solid-state nuclear magnetic resonance (NMR) spectroscopy can be used to probe the chemical environment of specific nuclei within molecules (Bugay, 1993). In NMR spectroscopy, the sample is exposed to a magnetic field that splits the energy levels of

nuclei having a net spin other than zero. The lower energy level nuclei are excited to the higher level by electromagnetic radiation at a specific frequency depending on the type of nucleus. The energy needed to excite the nuclei to the higher energy level is proportional to the magnetic field experienced by the nuclei, which is again dependent on the chemical environment of the nuclei. This causes a so called chemical shift in the absorbed frequency due to the shielding by electrons around the nucleus. The nucleus studied in the solid state is usually ^{13}C. The lack of random, averaging motion encountered in the liquid state and the long relaxation times of ^{13}C results in the need of various signal enhancing techniques in the solid-state applications (Bugay, 1993, 1995, 2001).

The solid-state NMR spectra show the differences in spatial positions of nuclei in different packing arrangements as a change in the isotropic chemical shift of the corresponding nuclei in each structure (Bugay, 2001). Hence, solid-state NMR spectroscopy can be applied to study polymorphism and amorphism. In addition, the technique provides information about the molecular motions occurring at the nuclei studied (Tishmack *et al.*, 2003). The pharmaceutical applications of solid-state NMR have been recently reviewed by Tishmack *et al.* (2003). It has been found more useful for the study of the amorphous state than X-ray diffraction (Tishmack *et al.*, 2003). An advantage of the technique is that the particle size has a very minor, if any, effect on the analysis (Bugay, 2001). The fact that it is not trivial to obtain high-quality spectra may be considered as a disadvantage (Tishmack *et al.*, 2003).

2.2.6 Microscopy

Optical and electron microscopy give information of the morphology of the crystals under study (Brittain, 1999). The morphology is of interest because the observable habits of different crystal structures are different. Polarization microscopy is based on the way the analyte crystal affects polarized light that is transmitted through the crystal at different angles (Newman and Brittain, 1995). The method can be utilized for the study of crystal systems (Emons *et al.*, 1982). There are seven different crystal systems: cubic, hexagonal, tetragonal, orthorhombic, monoclinic, triclinic and trigonal. The classification is based on relative lengths of lattice axes and the angles between the axes. The refractive index of the crystal in the direction of the different crystal axes is

dependent on the class the crystal belongs to. In some cases, the method can be also used to differ between amorphous and crystalline material. A distinctive advantage of a microscopic method is the extremely small sample amount necessary, thus, it can be a valuable technique in the early development stages.

2.2.7 Thermal methods

In thermal methods of analysis, a property of an analyte is studied as function of externally applied temperature (Giron, 1986). The two most common thermal methods applied in pharmaceutical sciences are *differential scanning calorimetry* (DSC) and *thermogravimetry* (TG). DSC records the heat flow in and out the sample. Thus, it can be used to analyse endothermic (melting, boiling, sublimation, vaporization, desolvation, glass transitions, chemical degradation, etc.) and exothermic (crystallization, oxidative decomposition) events (Brittain, 1999; Clas *et al.*, 1999). In addition, stability relationships (enantiotropy and monotropy) between different polymorphic forms can be studied using this method (Giron, 1995). In TG, the measured parameter is the weight loss of the material. This method is useful in the characterization of desolvation processes of hydrates and other solvates. For example, the stoichiometry of a solvate may be determined by this method.

Thermal microscopy is a technique which enables the visual inspection of changes in crystals as function of temperature (Kuhnert-Brandstätter, 1982). It can be used to investigate melting points and the desolvation of solvates. For example, thermal microscopy has been applied for the identification of channel hydrates (Byrn and Lin, 1976; Perrier and Byrn, 1982).

Mircocalorimetry is the measurement of heat flow (power, W) with time or temperature in micro-Watt scale (Gaisford and Buckton, 2001). In isothermal microcalorimetry, the power is measured as function of time at a specified temperature, whereas in scanning microcalorimetry, the power is measured as function of temperature. The latter is actually the same as DSC, but the instruments are highly sensitive, and thus, the method is referred to as high-sensitivity DSC. Low levels (1-2%) of amorphous material can be detected by isothermal microcalorimetry (Giron, 2001). The method is based on inducing crystallisation of the amorphous part in the chamber by moisture and measuring the heat of crystallisation. A limitation of this

method is that it can only be applied for amorphous materials which crystallise spontaneously under certain relative humidities or organic vapours (Gao and Rytting, 1997).

Processes involving enthalpy changes can also be investigated applying *solution calorimetry* (Giron, 1995). The difference in the heats of solution in any solvent will be equal to the difference in enthalpy of the solids, provided that the dissolution is rapid and no association or complexation takes place. It is possible to make quantitative determinations of the degree of disorder by this method (Gao and Rytting, 1997; Hogan and Buckton, 2000; Pikal *et al.*, 1978), but the method requires 100% crystalline and amorphous standards. The method allows also the quantification of polymorphs. An increasingly popular trend has been to combine the thermoanalytical techniques with microscopy, spectroscopy, XRPD or mass spectrometry (Giron, 2001).

2.3 Wet agglomeration

2.3.1 Agglomeration mechanisms

Agglomeration, also termed granulation, is a process where particles are brought together into larger semi-permanent aggregates, so called agglomerates or granules, where the original particles are still distinguishable (Snow *et al.*, 1997). In wet agglomeration, this process is facilitated by a liquid. The liquid binds the particles by a combination of capillary and viscous forces in the wet state (Iveson *et al.*, 2001). More permanent bonds are formed during subsequent drying. The aim of agglomeration is to improve powder flow and handling, decrease dustiness and prevent segregation of the API.

According to Iveson *et al.* (2001) there are fundamentally only three rate processes determining wet agglomeration behaviour: (1) wetting and nucleation; (2) consolidation and growth; and (3) breakage and attrition. These phenomena often take place simultaneously in the granulation equipment, making the investigation of the effect of an individual phenomenon on the agglomerate properties difficult.

Wetting of the particles is necessary for nucleation, i.e. the formation of initial agglomerates. The nucleation rate is governed by wetting thermodynamics and drop penetration kinetics (Hapgood *et al.*, 2002), as well as the binder dispersion. The binder

dispersion in the powder mass depends on the liquid delivery parameters (Knight *et al.*, 1998) and powder mixing (Litster *et al.*, 2001).

Agglomerate growth takes place whenever material in the granulation equipment collides and remains together. This is referred to as coalescence, when the colliding parties are two agglomerates, or as layering, when fine particles stick to the pre-existing agglomerates. The ability of two agglomerates to coalesce is dependent on many factors (Ennis *et al.*, 1991; Tardos *et al.*, 1997) including the strength and deformability of the agglomerates, and the availability of liquid in the proximity of their surfaces. Hence, liquid saturation is an important factor effecting agglomerate growth (Kristensen *et al.*, 1984). Liquid saturation can be increased either by increasing the liquid content or by consolidation of the agglomerates. The extent of consolidation depends on formulation properties and process variables. Moreover, the consolidation affects the strength and deformability of the agglomerates. The agglomerate strength is controlled by three factors: capillary, viscous and frictional forces, which are inter-related in a complex way (Iveson *et al.*, 2001). The relative importance of these forces can vary considerably with strain rate and formulation properties. On the other hand, deformation and breakage will take place, when the agglomerates reach a certain critical size, which depends on the applied kinetic energy and on the agglomerate strength (Tardos *et al.*, 1997). Summing up, agglomerate growth is dependent on many interrelated phenomena and determined by the balance between coalescence and breakage (Schaefer, 2001).

As noted above, breakage of wet agglomerates will affect and may control the final agglomerate mean size and size distribution (Iveson *et al.*, 2001; Tardos *et al.*, 1997). Breakage influences also the binder dispersion in the wetting and nucleation phase. Furthermore, attrition of dry granules leads to generation of dusty fines, which is undesirable.

2.3.2 Process monitoring of agglomeration in high-shear mixers

Wet agglomeration can be carried out in a high-shear mixer among other equipment. In this type of equipment, the particles are set into movement by an impeller (Fig. 5) rotating at a high speed. It contains also a chopper which breaks large aggregates. The binder liquid is added by pouring, pumping or spraying from the top. Wet agglomeration in a high-shear mixer involves typically six phases (Holm, 1997): First

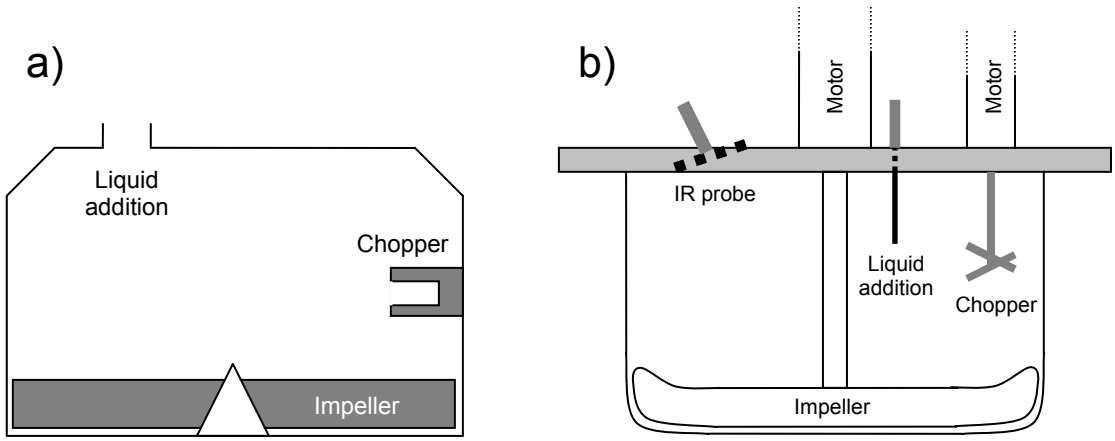

Fig. 5. Schematic diagrams of high-shear mixers: a) the main parts of a vertical high-shear mixer and b) the changeable bowl mixer used in the thesis.

the materials are dry mixed, where after liquid is added during mixing. Then the moist mass is wet massed in order to achieve a narrow particle size distribution. Thereafter the granules are wet sieved, dried and sieved again. The liquid amount is critical, because the process is susceptible for over-wetting, which leads to uncontrollable agglomerate growth. Variations in raw materials may affect the liquid requirement.

Impeller torque, (Lindberg, 1977) and power consumption (Bier *et al.*, 1979; Leuenberger *et al.*, 1979) of mixers have been used to monitor the properties of wet masses during agglomeration. The methods give a measure of the amount of resistance the impeller experiences to keep a certain rotational speed. It has been shown that these measurement techniques give the same information (Bier *et al.*, 1979; Corvari *et al.*, 1992; Mackaplow *et al.*, 2000), but direct torque measurements have been found to be the most sensitive (Kopcha *et al.*, 1992).

Leuenberger and co-workers (Bier *et al.*, 1979; Leuenberger *et al.*, 1979) used the power consumption curve during the liquid addition phase to find the optimal liquid amount for agglomeration. They divided the curve into different

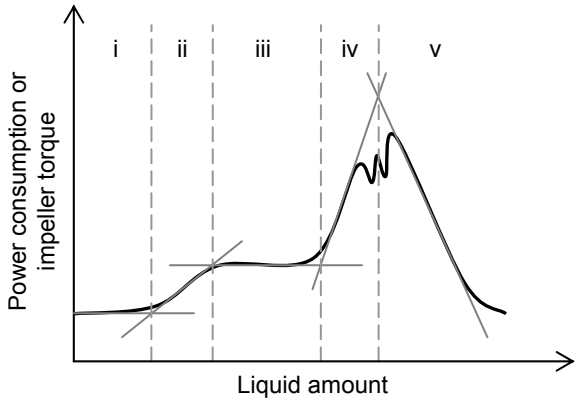

Fig. 6. A schematic presentation of a power consumption or impeller torque curve with the division to phases (see text for explanation).

phases by drawing tangents on the curves and using the intersections of these to mark the phase boundaries (Fig. 6). During the first phase the particles are wetted (i), where after the power consumption increases (ii) due to nucleation. Thereafter, the power consumption levels off to a plateau (iii), and then increases further (iv) in the fourth phase. During the last phase, the power consumption falls (v) as the mass becomes a suspension. According to Leuenberger the optimal liquid amount is located in the third phase.

If the power consumption curve is differentiated, the second phase is observed as a peak which can be used for process control (Holm *et al.*, 1985b; Leuenberger and Imanidis, 1986). From this peak, time is measured to reach the necessary water amount predetermined by experiments. However, the plateau phase is not observed for all materials disabling the peak detection method (Holm *et al.*, 2001).

The absolute values of power consumption are dependent on the formulation and the granulation equipment. Holm *et al.* (1985b) demonstrated a correlation between power consumption and granule growth. This relationship is although influenced by the process conditions (Holm *et al.*, 1985b) and equipment variables (Holm *et al.*, 2001). Several authors have pointed out that adhesion to the granulator bowl wall disturbs the power consumption or torque measurements (Holm *et al.*, 1985b; Lindberg, 1977; Mackaplow *et al.*, 2000). Examples on process control by power consumption measurements are given by Werani (1988) and Laicher *et al.* (1997).

It is still somewhat unclear which wet agglomerate properties the power consumption, or impeller torque, reflects mainly due to the complex nature of agglomeration. The power consumption has been related to cohesive forces arising from capillary pressure (Leuenberger *et al.*, 1979), to liquid saturation (Holm *et al.*, 1985a), to intragranular porosity (Ritala *et al.*, 1988), to interparticle friction forces (Pepin *et al.*, 2001), and to agglomerate tensile strength (Betz *et al.*, 2003; Holm *et al.*, 1985a; Leuenberger *et al.*, 1979), which displays the aforementioned factors. Pepin *et al.* (2001) speculated that the plateau phase arises from an increase in the energy dissipated by interagglomerate collisions due to increasing average size, and the reduction of the number of collisions due to the decreasing number of agglomerates, thus resulting in a constant level of power consumption.

Other process monitoring approaches have also been described for high-shear mixers. Vibrations of a probe located in the granulator have been related to the mass median diameter of granules (Ohike *et al.*, 1999; Staniforth *et al.*, 1986). The moisture distribution and packing of the mass has been followed by conductivity (Spring, 1983) and capacitive sensors (Corvari *et al.*, 1992; Fry *et al.*, 1984, 1987). By acoustic emission, sound produced by the process is detected and analysed. A correlation between the acoustic emission from a high-shear granulator and agglomerate size was found (Whitaker *et al.*, 2000). In a very different approach, Watano *et al.* (2001) introduced an image processing system for in-line measurement and control of the agglomerate size. Further, in an approach similar to torque measurements, stress fluctuations were used as input instead of average stresses (Talu *et al.*, 2001). However, the materials and methods used in that study were of model character and it is uncertain how applicable this technique is in monitoring the agglomeration of real materials.

2.4 Approaches to increase process understanding

2.4.1 Anticipation of processing-induced transformations

Many of the pharmaceutical unit-operations subject the active pharmaceutical ingredients to rather harsh conditions (Brittain and Fiese, 1999; Morris *et al.*, 1998, 2001; York, 1983). Examples of such unit-operations are milling, wet agglomeration, tabletting and lyophilization. Zhang *et al.* (2004) reviewed recently the potential phase transformations associated with common unit operations.

The unit-operations introduce some stress into the system (Morris *et al.*, 2001). The stress in this context is a physical change that moves the system from or towards equilibrium. The stress may be thermal, mechanical or a result from interaction with another factor like moisture. The system may be trapped in another equilibrium under the stress conditions producing a metastable form when the stress ceases or it may be kinetically formed to an everywhere metastable form. The stresses disturb the propagation of lattice vibrations in a crystal lattice. At low levels of stress, local strains develop in the lattice. If the local strain fields increase in number and size, they create larger and larger domains of strain until the lattice undergoes a global transformation, and a new phase is created with an own set of lattice vibrations.

Phase diagrams may be used to be able to overview the possible phase transitions which may take place during a process (Morris *et al.*, 2001). The physical conditions (temperature, pressure, humidity) the system encounters during the unit-operation and storage should be covered. The phase diagrams do however not show the probability of these possible transitions. The resulting phase after processing is governed not only by the most stable state in equilibrium but also by the kinetics of the transition and the time scale of the applied stress.

The kinetics of the transformation is governed by the activation energy associated with the transition (Giron, 1995). The rate of transition changes as function of temperature. The rate is minimal near the transition temperature (T_t) of two enantiotropic polymorphs and increases on both sides of the T_t. At low temperatures the rate decreases again. If the transition requires a major reorganisation of the structure, the metastable form may be stable in practice (e.g. diamond). One should be aware of that the presence of excipients (Airaksinen *et al.*, 2003) may affect the transition kinetics. In addition, the formulation matrix (Giron, 1995) or impurities (Bauer *et al.*, 2001) may induce the transformation into a more stable form, which does not otherwise take place. Changes in the time scale of the process due to scale-up should also be considered.

Wet agglomeration, with the subsequent drying process, is a unit-operation that includes several stresses, such as humidity, mechanical stress, and elevated temperatures, which may lead to processing-induced transformations. The granulation liquid used may take part in solution-mediated transformations (sometimes referred to as solvent-mediated), where the starting material transforms to a more stable polymorphic form or to a solvate (Morris *et al.*, 2001). In the drying process that follows wet agglomeration, the solvate may transform to a metastable or amorphous form. This can also be the case, if an ingredient has completely dissolved in the granulation liquid and precipitates during the drying process.

In this thesis, the solution-mediated transformations of theophylline and caffeine to their respective hydrates were under study. Wet granulation using water exposes these drugs to conditions where the hydrate is the stable form, because the water activity is increased remarkably by the granulation liquid. A solution-mediated transformation involves several steps. First, the metastable phase, in this context the anhydrous phase,

starts to dissolve. The solubility of the stable phase, in this context the hydrate, is lower than the solubility of the metastable phase, and, thus, the granulation liquid becomes supersaturated with respect to the stable phase. Thereafter, nucleation of the stable phase has to take place. It has been shown that the anhydrous theophylline crystals act as heterogeneous nucleation substrates for the hydrate phase, i.e. the hydrate crystals nucleate on the anhydrous phase (Rodríguez-Hornedo *et al.*, 1992). Heterogeneous nucleation lowers the free energy barrier compared to homogeneous nucleation, and this form for nucleation can occur at low driving forces (Rodríguez-Hornedo and Murphy, 1999). After the nucleation the stable phase continues to grow. This growth causes the concentration of the solution to fall. This leads to that the solution becomes under-saturated with respect to the metastable phase, which dissolves further. Therefore, the growth of the stable phase is maintained by the supersaturation created by the dissolution of the metastable phase. The process continues until the metastable phase has disappeared (Cardew and Davey, 1985).

The kinetics of solution-mediated transformations is governed by dissolution of the unstable phase and nucleation and growth of the stable phase. The dissolution of the metastable phase creates a supersaturation respect to the less soluble stable form and then acts as the driving force for the nucleation and growth of the stable phase. As the metastable phase and the excipients in the formulation act as nucleation sites for the stable phase, rapid nucleation can be assumed relative to the granulation time (Davis *et al.*, 2003). The overall kinetics can be either controlled by the dissolution of the metastable phase or by the growth of the stable phase (Cardew and Davey, 1985). Davis et al. (2003) presented a conceptual model for solution-mediated transformations during wet granulation.

2.4.2 Regulatory perspectives

The US Food and Drug Administration (FDA) has launched a Process Analytical Technology (PAT) initiative in order to encourage the industry to implement new technologies in the manufacture of drug products (FDA, 2003a,b). They define PAT as follows: “PAT is considered to be a system for designing, analyzing, and controlling manufacturing through timely measurements (i.e., during processing) of critical quality and performance attributes of raw and in-process materials and processes with the goal

of ensuring final product quality." In the construction of this framework, different parties such as pharmaceutical manufacturers and academia have been actively involved (FDA, 2003b).

In the initiative, the need for increased process understanding is stressed. A process can be generally considered well understood when all critical sources of variability are identified and explained, variability is managed by the process and the outcome of the process can be predicted over the ranges of material variability, process parameters and manufacturing conditions. PAT includes the optimal application of process analytical chemistry (PAC) tools, feed-back process-control strategies, information management tools, and product-process optimisation strategies to the manufacture of pharmaceuticals (Balboni, 2003). Advantages of implementing PAT would be reduced cycle times, prevention of rejects, reduction of human errors by automation, and facilitation of continuous processing to improve efficiency. Another gain of applying PAT would be that laborious testing of the finished product is avoided because the product can be released based on the in-process documentation. The European guidelines offer also this possibility calling the concept parametric release (EMEA, 2001). The benefits of implementation of PAT will vary depending on the product. Manufacturing of complex dosage forms as tablets will probably gain most (Balboni, 2003). The application of PAT to crystallization processes has been discussed recently (Yu *et al.*, 2004).

2.4.3 Process analytical technology tools

The main tools in the process analytical technology framework can be categorized as multivariate data analysis tools; process analytical chemistry tools; process monitoring and control tools; and continuous improvement and knowledge management tools (FDA, 2003a).

Multivariate data analysis tools To effectively use the large body of data collected during a development program, means are needed in order to extract the relevant information from the large amount of data collected. In addition, traditional one-factor-at-a-time experiments do not effectively reveal interactions between product and process variables. Chemometric techniques can be applied for multivariate data analysis and design of experiments. Chemometrics can be defined as the science of

relating measurements made on a chemical system to the state of the system via application of mathematical and statistical methods and computer sciences (Geladi and Dåbakk, 1995; Wise and Kowalski, 1995). There is a myriad of chemometric methods available. The methods can be roughly divided into (Beebe *et al.*, 1998; Brereton, 2003) experimental design frameworks; signal pre-processing techniques; dimension reduction tools, such as principal components analysis (PCA); and multivariate calibration and prediction methods, such as partial least squares (PLS). The chemometric tools in conjunction with knowledge management tools can be used to define the product and process variables that are critical to product performance. In the following, the chemometric tools used in this thesis are presented.

The most popular linear projection method in chemistry is principal components analysis (PCA). It allows projection of multivariate data into a few new variables, which maximize the capture of data variance. The original data matrix, X (I x J matrix) is decomposed to scores, T (I x A matrix) and loadings, P^T (A x J matrix) (Fig. 7). The scores describe the variation in the data and the loadings describe how the principal components are related to the original variables. The residual matrix, E, contains the part not modelled by the scores and loadings i.e. the noise.

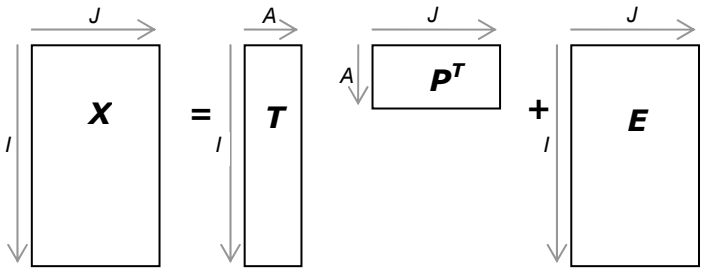

*Fig. 7. A schematic description of principal components analysis. The original data matrix (*X*) is decomposed to scores (*T*) and loadings (*P^T*). The residual matrix (E) is the part of* X *which the scores and loadings do not explain. The arrows present the dimensions of the matrixes and A is the number of principal components extracted. Modified from Grung (1996) and Brereton (2003).*

The decomposition can be written as

$$X = t_1 p'_1 + t_2 p'_2 + \ldots + t_A p'_A + E \qquad (11)$$

where t denotes the score vector, p the loading vector, A the number of principal components extracted and E the residuals which are not described by the model.

Another projection method, which is conceptually close to PCA, is the self-organizing map (SOM). The main difference between these methods is that the SOM is

non-linear. The SOM is a map of nodes bound to their neighbours by elastic bonds. The trained SOM is ordered in such a way that data clusters with similar properties are located near each other. In an n-dimensional data space, if the input vector is denoted by $x = [x_1, x_2, ..., x_n]^T$ and the location vector of a mapping node by $m_i = [m_{i1}, m_{i2}, ..., m_{in}]^T$, the algorithm describing the self-organizing operation can be written as follows (Kohonen, 1997):

I Initiate the locations of nodes with random values.

II For each vector of the training data compute steps IIIa and IIIb,

IIIa find the SOM node m_c best matching to the data vector $x(t)$ by searching all nodes m_i

$$\|x(t) - m_c(t)\| = \min_i \{\|x(t) - m_i(t)\|\} \tag{12}$$

IIIb adjust the locations of the nodes

$$m_i(t+1) = \begin{cases} m_i(t) + \alpha(t)\{x(t) - m_i(t)\}, & \text{for } i \in N_c \\ m_i(t), & \text{for all other indices} \end{cases}. \tag{13}$$

In Eq. (13) N_c refers to a neighbourhood set of array points around the node c. The Euclidean metric can be used as the distance measure in Eqs. (12) and (13). The parameter $\alpha(t)$ in eq. (13) defines the learning rate, i.e. how much the winning node and its neighbourhood are moved to the direction of the data vector $x(t)$, and it should decrease slowly with time. A diagram on the architecture of the map is presented in Fig. 8.

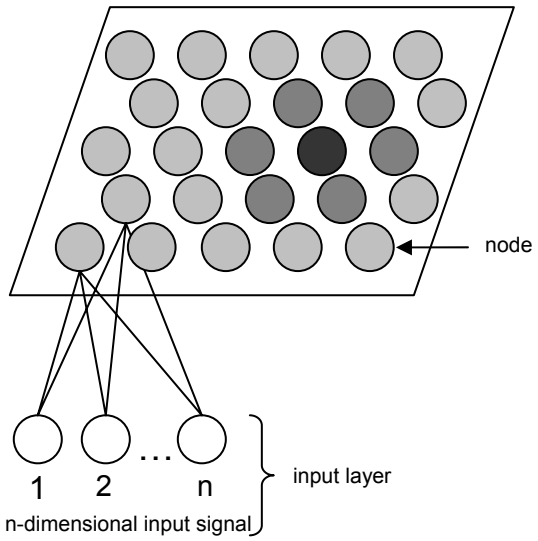

Fig. 8. The self-organising map architecture. The black neuron depicts a best matching unit and the dark neurons around it show the neighbourhood.

Process analytical chemistry tools The objective of process analytical chemistry (PAC) is to obtain quantitative and qualitative information about a chemical process (Callis *et al.*, 1987). This information can be used to monitor, control and optimise processes in terms of capacity, quality, cost, consistency and waste reduction (McLennan, 1995). The available PAC tools have developed from simple process measurements, such as pH, temperature and pressure, to ones that measure chemical and physical attributes of the product.

Different possibilities exist to perform measurements from a process (Callis *et al.*, 1987; McLennan, 1995). These different ways of analysis can be defined as follows: *Off-line*, manual sampling with transport to a centralised laboratory; *at-line*, the analyser is located in close proximity of the process; *on-line*, the sampling and the analysis are automated; *in-line*, the analyser probe is located in the process stream; and *non-invasive*, the measurement is performed using a probe which is not in contact with the process stream (Callis *et al.*, 1987). Other authors treat the last three concepts as one group (McLennan, 1995).

Process monitoring and control tools Process monitoring and control strategies offer the possibility to monitor the state of the process and to actively change the process variables to maintain a desired state. After identifying the critical material and process attributes related to product quality, systems enabling the real-time or near real-time measurement of these should be designed. Mathematical relationships between these measurements and the product quality attributes should then be developed. Further, process controls that actively adjust the process in order to achieve the desired process outcome should be created. Then, it should be possible to determine process end-points by achievement of the desired product or in-process material attributes.

Spectroscopic methods such as diffuse reflectance NIR and Raman can be applied for real-time measurements. NIR has been applied to process monitoring of production of API (Norris *et al.*, 1997; Norris and Aldridge, 1996), nanoparticle production (Higgins *et al.*, 2003), powder blending (Hailey *et al.*, 1996; Sekulic *et al.*, 1996; Ufret and Morris, 2001), fluid bed granulation (Frake *et al.*, 1997; Goebel and Steffens, 1998; Rantanen *et al.*, 1998), coating (Andersson *et al.*, 1999; Andersson *et al.*, 2000; Kirsch and Drennen, 1996), drying (Harris and Walker, 2000; Zhou *et al.*, 2003) and

lyophilization (Brülls *et al.*, 2003; Last and Prebble, 1993). Raman spectroscopy has been utilized in in-line synthesis monitoring (Svensson *et al.*, 2000) and *in-situ* monitoring of polymorph conversion (Starbuck *et al.*, 2002) of APIs, and in end-point detection of blending (Vergote *et al.*, 2004).

Continuous improvement and knowledge management tools It is emphasized in the PAT strategy, that continuous learning throughout the life-time of a product is important. This can be achieved through continuous data collection and analysis using information technology systems that allow knowledge acquisition from process and other databases. Continuous improvement also requires increasing cooperation between formulation, process development and manufacturing organisations. In addition, the information technology systems should be designed in a way that allows all these organisations an access to the PAT data and already existing data systems, and enables the compilation of data in a straight forward manner (Neway, 2003). Otherwise the full potential of the large amounts of data gathered by PAT will be lost. The lack of real-time data availability with a connection to the end-point user may be one of the largest obstacles to better compliance and operation efficiencies.

3 AIMS OF THE STUDY

The overall aim of the present study was to elucidate the use of spectroscopic methods as means to gain process understanding in wet agglomeration. The wet agglomeration process was studied from two different aspects. On one hand, the impact of the process on the solid-state of the active pharmaceutical ingredient was studied and, on the other hand, the progress of agglomeration itself was looked into in high-shear mixers. As the spectroscopic and other process analytical methods produce a large amount of data, dimension reduction tools were studied as well. The specific aims were:

- to study anhydrous to hydrate transformations of two structurally related model drugs during wet agglomeration using near-infrared and Raman spectroscopy
- to investigate the effect of excipients on hydrate formation of theophylline
- to compare near-infrared spectroscopy with an established method, impeller torque measurement, in the characterization of wet agglomeration
- to get fundamental understanding by combing information obtained by different measurement techniques in the visualisation of wet massing phase of high-shear granulation
- to investigate two visualisation approaches, a linear and a non-linear, in the visualisation of the combined granulation process data.

4 EXPERIMENTAL

Detailed descriptions of the materials and methods can be found in the respective papers (I-VI).

4.1 Materials

Anhydrous theophylline (form II) from BASF (Ludwigshafen, Germany) (I, II, V, VI) and Orion Pharma (Espoo, Finland) (III, IV) and anhydrous caffeine (low temperature form) from Orion Pharma (Espoo, Finland) (II) were used as model drugs. α-Lactose monohydrate (Pharmatose 200M, DMV International, Veghel, the Netherlands) (III-IV), silicified microcrystalline cellulose (Prosolv 50, Penwest Pharmaceuticals, Nastola, Finland) (III, IV), and microcrystalline cellulose (Avicel PH-101, FMC International, Little Island, Cork, Ireland) (V, VI) were used as excipients.

Theophylline monohydrate (I, II) and caffeine 4/5 hydrate (II) were prepared by dissolving the anhydrous form in hot (60 and 80 °C, respectively) purified water and cooling the solution slowly. The crystals were harvested by vacuum filtering. The crystals were stored at 58% relative humidity produced by a saturated salt solution (NaBr). In addition, theophylline monohydrate was prepared (V) by spreading the anhydrous theophylline as a thin layer on metal trays and storing these in 30 °C and 92%RH for at least 8 days, in order to obtain a particle size as near as possible as that of the anhydrous theophylline.

4.2 Characterisation of primary materials

Moisture contents were measured by loss on drying using an IR dryer: Sartorius Thermocontrol (YTC01, Sartorius GmbH, Göttingen, Germany (I, II)) or Mettler Toledo Moisture Analyzer (HR73, Mettler Toledo, Geifensee, Switzerland (V, VI)); and by Karl Fisher titration (III) (Mettler Karl Fisher titrator, D35, Mettler Toledo, Geifensee, Switzerland).

The pycnometric density (V, VI) was measured using helium pycnometry (Accupyc 1330, Micromeritics, Norcross, GA, USA). The volume size distribution (V, VI) was measured by performing aerodynamic time of flight (Aerosizer, TSI Inc., St. Paul, MN, USA) and laser diffraction (Malvern Mastersizer S, Worcestershire, UK)

measurements. The poured and tapped densities (V, VI) were measured according to Ph.Eur. 4th Ed.

Contact angles (V, VI) were measured using Krüss Contact angle measuring system (G2, Krüss GmbH, Hamburg, Germany). Compacts of both materials were made using a hydraulic press (Perkin-Elmer GmbH, Überlingen, Germany) compressing with a pressure of approx. 500 MPa for 2 min. Water (Milli-Q grade) was dropped on the compacts and the contact angle was determined from a video with circle fitting method during the first second after drop impact.

The pycnometric density measurements were performed in duplicate, the contact angle measurements five times and the other characterization measurements in triplicate.

4.3 Processing of materials

4.3.1 Preparation of wet masses

Wet masses of anhydrous theophylline (I, II) and anhydrous caffeine (II) were prepared using a planetary mixer (Kenwood KM400, Kenwood Ltd., UK). The granulations were carried out by adding five different amounts of purified water at a constant speed into 300 g of anhydrous material with a low agitation speed (60 rpm) and mixing for 5 minutes with a higher speed (120 rpm) after water addition. The water amounts used were 0.3, 0.7, 1.3, 2.0 and 2.7 moles of water per mole of anhydrous material for theophylline and 0.3, 0.6, 0.9, 1.5 and 2.1 moles of water per mole of anhydrous material for caffeine.

Wet masses of anhydrous theophylline and 1:1 (w/w) mixtures with the excipients were prepared (III, IV) using the planetary mixer. A batch size was 500 g of starting material, and 0.03, 0.07, 0.10, and 0.20 g g^{-1} (III) or 0.10 g g^{-1} (IV) of purified water was added at 200 g min^{-1} using a pump. The masses were mixed for 5 min (III) or 2-3 min (IV) after the addition of water.

For at-line analysis, a wet mass of anhydrous theophylline was prepared in the planetary mixer (II). 3 or 6 ml purified water was added into 300 g anhydrous theophylline while mixing with a low speed (60 rpm) and then wet massing with a higher speed (120 rpm) for 30 s. Two samples were taken to immediate NIR analysis

and put back in the mass after analysis. The addition of water was repeated so that a range of 0.2-3.0 moles of water per mole of anhydrous theophylline was covered with 0.1-0.2 increments.

4.3.2 Granulation

A small-scale laboratory high-shear mixer (Mi-Pro, Pro-C-epT, Zelzate, Belgium) with a glass bowl of 1900 ml was employed in the granulation (V, VI). Torque of the impeller and temperature of the mass (with an IR probe) were recorded with 1 s intervals. Granulation liquid was added using a precision pump (Dosimat 765, Metrohm Ltd., Herisau, Switzerland) through a metal pipe. The precision pump employed in the granulator was limited to 50 ml and the liquid addition was paused for 20 s during the pump fill up, if larger quantities were needed.

Anhydrous theophylline, α-lactose monohydrate, microcrystalline cellulose (MCC) and mixtures of these (1:1 and 1:1:1, w/w) were granulated with purified water. The loads of the different masses were chosen so that the mass filled approx. ¼ of the bowl. Impeller and chopper speeds were 1000 and 2000 rpm, respectively. The masses were premixed for 3 min where after liquid addition was started. The liquid addition rate was chosen so that the masses became overgranulated (the impeller torque decreased steeply) around 3 min from the beginning of liquid addition. The process was stopped when the impeller torque dropped. All the mixtures were granulated in triplicate, except for the theophylline – α-lactose monohydrate wet mass which adhered severely to the bowl wall and hindered the granulation.

Another set of granulations with the same process conditions was performed with sampling for NIR measurements. Samples of 2-4 ml were taken with a spoon in closable plastic bags at 30, 60, 90, 120 and 150 s after the beginning of liquid addition, and at the end of the process. The process was paused for 30 s during sampling. Granulation of the tertiary mixture was performed five times and the others were granulated twice.

The effect of hydrate formation on the impeller torque curve (V) was investigated by granulating theophylline monohydrate with the same process conditions as anhydrous theophylline. The granulation was performed in duplicate. In one of the granulations a sample was taken at the end of the dry mixing phase and analysed by NIR spectroscopy, which showed that the material had not dehydrated.

In order to evaluate formation of granules visually, the α-lactose monohydrate – MCC (V) and the tertiary mixture (VI) was granulated and samples were withdrawn as described previously. A similar granulation was performed with the α-lactose monohydrate – MCC mixture where samples were taken at 45, 75, 105, 135 and 165 s after the beginning of the water addition and at the end of the process. The samples were dried at 40 °C for 24 h. The dry samples were evaluated by taking SEM images.

4.3.3 Mixer torque rheometry

The rheological properties of anhydrous theophylline and theophylline monohydrate wet masses were studied (V) using a mixer torque rheometer (Caleva Process Solutions Ltd, Dorset, England, UK). The temperature was set to 27 °C. 20 g of the dry powder was dry mixed for 3 min. 1.8 ml of purified water was added with a pipette during 10-15 s with the blades running. The mass was mixed for 1 min where after the torque was logged for 20 s. The water additions were repeated 7 times (total 12.6 ml). All the measurements were performed in triplicate. The MTR measurements were additionally performed using fractionated coconut oil (Miglyol 812, Sasol, Germany) instead of water.

4.4 Characterization of wet masses and granules

Three samples were taken from the wet masses for off-line analysis by near-infrared spectroscopy (I-III), Raman spectroscopy (II), XRPD (I-III) and DSC (I). The samples were measured the same day they were prepared (I, II) or after overnight equilibration (III).

In paper IV, four samples were taken from the masses into glass vials (20 ml) for NIR analysis and the rest was stored in well-closed plastic bags for sampling for X-ray powder diffraction (XRPD). The set of four samples was measured consecutively at various times after the wet massing. For XRPD, a new sample was taken at each time point.

The samples taken during granulation (V, VI) were moved into 4 ml glass vials for NIR at-line analysis. A diffuse reflectance (log 1/R) NIR spectrum of each sample was measured 20 and 27 min after the sampling from the granulation process.

Moisture contents were measured by loss on drying using a IR dryer: Sartorius Thermocontrol (YTC01, Sartorius GmbH, Göttingen, Germany (I-III)) or Mettler Toledo Moisture Analyzer (HR73, Mettler Toledo, Geifensee, Switzerland (V, VI)).

Diffuse reflectance near-infrared (log 1/R) spectra were obtained using a Fourier transform spectrometer (Bomem MB-160 DX, Hartman & Braun, Quebec, Canada) (I-IV). In papers V and VI, the spectra were measured using a FOSS NIRSystems spectrometer (Rapid content analyser, model 6500, FOSS NIRSystems Inc., Silver Springs, MD, USA). Second-derivative transformations with Savitzky-Golay smoothing (Savitzky and Golay, 1964) were computed using Grams/32 software (v. 4.04, Galactic Industries Corp., Salem, NH, USA) (I) or Matlab (v. 5.3, The MathWorks Inc., Natick, MA, USA) (II-IV, VI). Baseline correction (V, VI) of the OH stretching band (1^{st} overtone) at 1450 nm and of the OH combination band at 1930 nm was performed in Matlab (v.5.3, MathWorks Inc., Natick, MA, USA). The slope of the spectrum baseline (V, VI) was calculated by fitting a line through three points in the spectra by least squares fitting method. The points were at 1300, 1852 and 2180 nm for samples containing theophylline and at 1300, 1852 and 2256 nm for the rest of the samples.

Raman spectra were measured (II, III) with a charge-coupled device (CCD) Raman spectrometer prototype (VTT Electronics, Oulu, Finland). The spectral intensities were normalised by standard normal variate transformation (Barnes *et al.*, 1989; Svensson *et al.*, 1999) using Matlab (v. 5.3, The MathWorks Inc., Natick, MA, USA).

X-ray diffraction patterns were measured using an x-ray powder diffraction theta-theta diffractometer (Seifert XRD 3000, Rich. Seifert & Co., Germany, I) or a similar equipment (Bruker AXS D8, Bruker AXS GmbH, Karlsruhe, Germany, II-IV).

Images of the raw materials and dried granules were obtained using scanning electron microscopes (DSM962, Zeiss, Oberkochen, Germany (III)); (Jeol JSM 5200, Tokyo, Japan (V, VI)). The samples were coated with platinum (III) or gold (V, VI).

Differential scanning calorimetric (DSC) measurements (I) were carried out on a Mettler DSC analyser (model 821^{e}, Mettler Toledo Ag, Schwerzenbach, Switzerland). The heating rate was 10 °C min^{-1} over a temperature range of 25-300 °C.

4.5 Data analysis

4.5.1 Combination of process data to a process vector

Vectors were constructed from the process and spectral data (VI) describing the state of the process at each sampling time point. Data logged by the high-shear mixer was used: added water amount, impeller torque and increase in product temperature. The baseline-corrected water absorbance peak height at 1930 nm was used to follow the water amount. Ratios (D) of two second-derivative spectral wavelengths were used:

$$D = \frac{d^2\left[\log\left(1/R\right)_{\lambda_1}\right]}{d^2\left[\log\left(1/R\right)_{\lambda_2}\right]} \tag{14}$$

where the wavelength of interest was divided by another wavelength normalizing scattering effects (Luner *et al.*, 2000). The second derivative wavelengths were chosen by performing a principal component analysis (Simca-P 8.0, Umetrics AB, Umeå, Sweden). The nominator wavelengths were chosen from wavelengths with high loadings (explaining most of the variation in the data) and the denominators were chosen from the wavelengths with loadings near zero. These ratios were as follows: d_{1154}/d_{1498}, OH combination band of water; d_{1414}/d_{1498}, 1st overtone of OH; d_{1668}/d_{1702}, CH stretch of theophylline; d_{1902}/d_{2114}, OH combination band of free water; d_{1970}/d_{2114}, OH combination band of theophylline hydrate water. In addition, the slope of the spectrum baseline was used in the vector. These combined data vectors were then analysed using PCA and SOM.

4.5.2 Principal components analysis

The at-line NIR data from the water addition to theophylline (II) and the combined process data (VI) was analysed using principal components analysis (Simca-P, 8.0, Umetrics AB, Umeå, Swden).

4.5.3 Self-organizing maps

The combined process data (VI) was analysed also using self-organizing maps (SOM). The SOM computation was run in Matlab (v. 5.3, MathWorks Inc., Natick, MA, USA) using a public domain toolbox (SOM toolbox for Matlab, 2003) and default settings.

5 RESULTS AND DISCUSSION

5.1 Hydrate formation during wet granulation

In order to create better process understanding, the PITs possibly taking place should be understood and under control. Therefore, means are needed which can detect these phenomena and which can be used to follow the events real-time. Wet granulation is probably one of the harshest processes used in the production of pharmaceuticals in terms of moisture and heat and, therefore, offers conditions that favour PITs. Hydrate formation is a PIT which can take place during this process. The model drugs used in this thesis, theophylline and caffeine, are examples of substances that exhibit this kind of behaviour (Bogardus, 1983; Herman *et al.*, 1988). Many of the methods traditionally used for the characterisation of hydrates do not enable real-time measurements as NIR and Raman spectroscopy do. In this chapter, the use of NIR and Raman spectroscopy in detecting hydrate formation and the effect of excipients on this is discussed. In addition, the information obtained of the behaviour of the model drugs by these methods is touched upon. Moreover, the effect of hydrate formation on the granulation liquid requirement is discussed.

5.1.1 Detection of hydrate formation during granulation

The hydrate formation of theophylline (I) and caffeine (II) during granulation was detected using near-infrared spectroscopy. With increasing water content, absorbance maxima were observed at around 1475 and 1970 nm for theophylline and at around 1460 and 1960 nm for caffeine. These maxima could be related to the hydrate water. At large water amounts, free water absorbances (Choppin and Violante, 1972; Fornés and Chaussidon, 1978; Iwamoto *et al.*, 1987) were observed at around 1410 and 1905 nm. The hydrate water absorbance could be separated from the free water absorbance by carrying out a second-derivative transformation. It was also possible to detect the hydrate formation by Raman spectroscopy (II). Upon hydrate formation several bands shifted in the Raman spectra of the model substances. However, the vibrations of water were not generally detected as water is a weak Raman scatterer, but instead the changes taking place in the vibrational state of the drug molecules.

The kinetics of solution-mediated transformations are usually investigated by measuring the concentration profile of the transforming drug in solution (Davey and Garside, 2000; Davey *et al.*, 1986). This is however difficult in wet masses where the amount of liquid is small. It was possible to follow the rate of transformation by NIR spectroscopy in wet masses of theophylline (IV).

When creating a process analytical method it is necessary to investigate how the matrix, i.e. other substances in the product, affects the signal collected. Hence, the effect of two common excipients was studied. α-Lactose monohydrate and silicified microcrystalline cellulose (SMCC) did not interfere with the detection of hydrate formation using either of the methods (III). The hydrate water of lactose and the absorbed water in the SMCC were observed at different wavelengths in the 2nd derivative spectra than the hydrate water of theophylline, thus not interfering with the detection of theophylline hydrate water absorbance. The excipients had minor or no Raman peaks at the regions of interest. In addition, the Raman intensity of theophylline was much higher than that of the excipients.

According to the NIR results the conversion to monohydrate was completed at lower water amounts (I) or at earlier time points (IV) than to XRPD results. These discrepancies might be explained by differences in the effective sample sizes of the methods. NIR spectroscopy is a surface method, whereas x-rays penetrate throughout the sample. Various NIR information depths have been reported for pharmaceutical materials ranging from 100 μm (Andersson *et al.*, 1999) to 500 μm (Hammond *et al.*, 1999). Different materials exhibit different information depths due to variation in particle size, chemical composition and degree of compaction (Berntsson *et al.*, 1998; MacDonald and Prebble, 1993). The effective sample size is also dependent on the wavelength (Berntsson *et al.*, 1998). The small effective sample size can impede the use of NIR quantitatively.

5.1.2 Comparison of Raman and near-infrared spectroscopy

It was possible to follow the hydrate formation by both methods (II, III). The NIR spectroscopy enables the determination of the state of water and has also been used for quantitative determination of moisture (Osborne *et al.*, 1993). However, the prominent OH absorption may dominate the spectra, so that the changes in other vibrations

originating from other constituents are obscured. In addition, if the water content is high, such as in granulation with water absorbing excipients, the free water absorbance may overlap the hydrate water absorbance (Fig. 9). In this case, the original, not transformed water absorbance maximum (not shown) becomes so broad and blunt that it seems in the second-derivative spectra that the hydrate amount would decrease instead of increasing. A similar observation has been reported when studying hydrate formation during extrusion-spheronisation (Laitinen *et al.*, 2004). This problem is not encountered using Raman spectroscopy, as water does nearly not affect the spectra at all. Raman spectroscopy has been successfully used in quantitation of hydrates during wet massing and in aqueous slurries (Rantanen *et al.*, 2004).

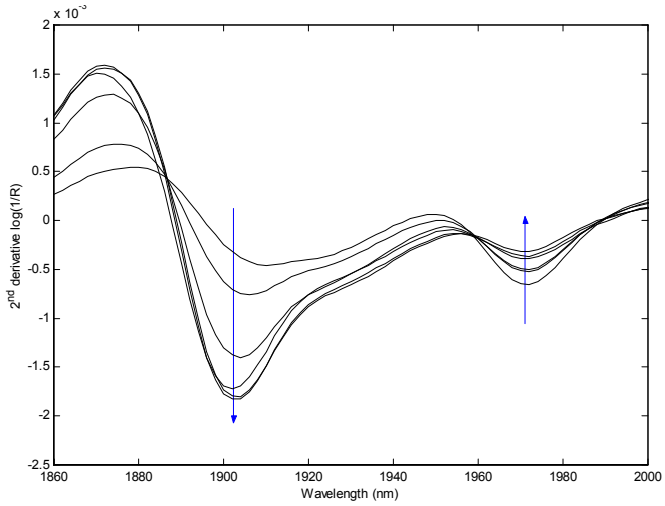

Fig. 9. Second derivative NIR spectra of theophylline - microcrystalline cellulose (1:1, w/w) wet granules. The water content ranges from 0.17-0.86 ml g^{-1}. Absorbance maxima are observed as troughs. Free water: 1905 nm, hydrate water: 1970 nm. Arrows indicate changes taking place with increasing water content. (Data from publication V).

Due to the broad and overlapping peaks and strong effect of the physical appearance of the samples in NIR spectroscopy, multivariate calibration is necessary in most cases, although univariate calibration models with adequate accuracy and precision have also been presented (Patel *et al.*, 2001). The peaks are better resolved in Raman, making it easier to develop a univariate calibration model. Multivariate calibration is often more straightforward to develop than univariate, but care has to be taken to select a sufficient amount of calibration and validation samples displaying all the possible variability encountered in the systems under study. If there is a slight change in the raw materials (e.g. new impurities, different particle size), the probability that this will affect the accuracy and precision of a multivariate method is greater than for a univariate method.

5.1.3 Differences between theophylline and caffeine

Theophylline and caffeine form similar channel hydrates (Sun *et al.*, 2002; Sutor, 1958a,b), caffeine having a larger channel cross-sectional area (Perrier and Byrn, 1982). There were smaller differences in the Raman spectra of caffeine and its hydrate than between those of theophylline and its hydrate (II). This suggests that a larger rearrangement takes place during hydrate formation of theophylline than during that of caffeine. The OH combination band of caffeine 4/5-hydrate (1960 nm, II) was observed at a shorter wavelength than that of theophylline monohydrate (1970 nm, I). The respective force constants, calculated using Eq. (7), are 1454 and 1439 Nm^{-1}. This indicates that the average strength of caffeine 4/5-hydrate OH bonds is greater than that of theophylline monohydrate. The caffeine hydrate is less stable than the theophylline hydrate, caffeine having lower activation energy of dehydration (Perrier and Byrn, 1982). It has been suggested that only a fraction of the water molecules located in the caffeine hydrate channel are hydrogen bonded to the caffeine, whereas the water molecules in theophylline hydrate are firmly linked to the theophylline molecules (Edwards *et al.*, 1997; Sutor, 1958b). This is probably reflected in the force constants.

Anhydrous theophylline transformed to a hydrate at smaller water amounts than anhydrous caffeine (I, II), indicating that anhydrous caffeine is more stable during wet granulation. The caffeine 4/5-hydrate is unstable at ambient conditions, and its dehydration rate increases when the particle size is small (Griesser and Burger, 1995). The vigorous mixing during granulation does probably not allow growth of large crystals. Therefore, caffeine 4/5-hydrate crystals are likely to partially dehydrate, although there would theoretically be enough water for all the caffeine to transform into hydrate. The DSC dehydration endotherm of theophylline was located at a lower temperature for the granules than for the long crystals crystallized from water (I), indicating that the hydrate formed during wet granulation is more likely to dehydrate than one crystallised from water. Moreover, it cannot be ruled out that the difference might be partially related to the kinetics of the transformation; the kinetics of caffeine being slower (Rantanen *et al.*, 2004).

5.1.4 Effect of excipients on hydrate formation

The API is usually present in an excipient matrix in pharmaceutical solid dosage forms. The excipients that exhibit water absorbing capability might have an effect on the hydrate formation of drugs. For example, it has been found that MCC affects the hydrate formation of anhydrous lactose (Angberg *et al.*, 1991) and polyvinylpyrrolidone protects theophylline against hydrate formation (Kesavan and Peck, 1996) in different relative humidities. Thus, the effect of excipients on the extent and kinetics of hydrate formation was studied. Two physico-chemically different excipients were chosen for the study: SMCC and α-lactose monohydrate.

MCC[1] is a porous material capable of taking up significant amounts of moisture (Fielden *et al.*, 1988; Zografi and Kontny, 1986). The absorbed water may exist in at least three different thermodynamic states in the amorphous region of MCC: directly bound to hydroxyl groups, as free water and in (an) intermediate state(s) (Zografi, 1988; Zografi and Kontny, 1986). SMCC was able to inhibit the formation of theophylline monohydrate in wet masses with low water contents (III). However, the inhibitory effect was not observed at water contents ≥ 0.1 g g^{-1} in the samples equilibrated overnight (III). SMCC slowed down the hydrate formation rate of theophylline at a water content of 0.1 g g^{-1} (IV). The absorbing process of SMCC might be faster than the hydrate formation, but then again, the hydrate formation might be thermodynamically favoured. This would explain the lag time and the retarding effect of SMCC, and the lack of the inhibiting effect at the end, respectively. Airaksinen et al. (2003) studied the effect of a wide range of excipients on hydrate formation of nitrofurantoin in wet masses. They found that low-substituted hydroxylpropyl cellulose inhibited totally the hydrate formation even at relatively high moisture contents (4 g g^{-1} of added water).

α-Lactose monohydrate is a crystalline material, characterized by its high aqueous solubility. The presence of α-lactose monohydrate in the wet masses increased the amount of hydrate formed (III) and seemed to increase the kinetics of the hydrate formation (IV) as well at a low water content. The origin of this slight, enhancing effect was not found, although it was speculated that it might be due to faster wetting (III, IV).

[1] The interactions of MCC and SMCC with water have shown to be similar (Buckton *et al.*, 1999; Luukkonen *et al.*, 2001); hence, literature concerning MCC is used to explain the results.

5.1.5 Effect of hydrate formation mechanism on liquid requirement of wet granulation

It was observed that theophylline monohydrate, which was prepared by hydration in high relative humidity, became over-granulated with a smaller quantity of water than anhydrous theophylline (V). The difference in liquid requirement was approximately 0.1 ml g^{-1}, when the theoretical amount of water taking part in the hydrate formation of anhydrous theophylline was taken into account. The difference observed using the high-shear granulation equipment was confirmed by mixer torque rheometry. A similar difference was perceived in the liquid amount needed for reaching the maximum torque value of the two forms. The product temperatures of anhydrous theophylline in the high-shear mixer were about 4 °C higher than that of theophylline monohydrate, suggesting that more evaporation could have taken place in the anhydrous theophylline masses. However, the temperature difference was minor (0.6 ± 0.1 °C) in the MTR measurements, indicating that this was probably not the explanation.

Different powder properties, which are related to the granulation liquid requirement, were evaluated, in order to explain the observed difference (V). Particle size affects the liquid requirement, as smaller particles having a larger surface area require more liquid. Although problems were encountered with the particle size measurements, the particle sizes of the two powders were evaluated to be so similar, that it is unlikely that this would have such a large impact on the liquid requirement. Moreover, the liquid requirement is increased by poor wetting. The smaller contact angle of anhydrous theophylline indicated a smaller liquid requirement in contrast to the results. Furthermore, attempts were made to measure the specific surface area of the two theophyllines. Unfortunately, the monohydrate dehydrated during the sample preparation, making the measurements of theophylline monohydrate impossible.

Thus, it was assumed that the phase transition occurring during granulation might cause the difference. The MTR mean torque curves were almost identical when the experiments were performed using fractionated coconut oil instead of water as the liquid (V). This result indicates that the phase transition from anhydrous theophylline to theophylline monohydrate during granulation was the cause of the increased liquid requirement.

5.2 Monitoring wet granulation in a high-shear mixer

The only high-shear granulation process monitoring strategies, which have won wider popularity, are power consumption and impeller torque measurements. These techniques have although some drawbacks. They are affected by equipment and process variables (Holm *et al.*, 2001). In addition, adhesion to the bowl wall may create irreproducible results (Lindberg, 1977; Mackaplow *et al.*, 2000). It has been also mentioned that hydrate formation during granulation disturbs the measurements (Bier *et al.*, 1979), but it has not been presented how. Hence, new methods would be desirable. NIR spectroscopy is a fast method, which is capable of detecting both chemical and physical changes in powder samples. Thus, it may be a potential method for process monitoring of high-shear granulation. In this chapter, one of the standard methods describing the state of *wet* agglomerates, namely the impeller torque, is compared to data obtained by at-line NIR measurements. In addition, the effect of hydrate formation on the impeller torque is discussed.

5.2.1 Near-infrared spectroscopy versus impeller torque

The use of the NIR spectra in reflecting agglomerate properties was investigated from two different aspects: using baseline corrected water absorbances, which expressed the water amount in the system, and the slope of the spectrum baseline, which reflected changes in the physical properties of the samples. These outputs were compared to the impeller torque, which use is well described in the literature. The impeller torque curves of the different materials and their mixtures had the typical shape reported earlier (Bier *et al.*, 1979), save α-lactose monohydrate (V).

Non-linearity was observed in the baseline corrected water absorbances (V). The hydrate formation of theophylline resulted in an increasing slope of the water absorbances at the used wavelengths. The slope of the baseline corrected water absorbance at 1930 nm of MCC and the MCC mixtures declined at the torque plateau, i.e. at the optimal water amount region stated by Leuenberger and co-workers (Bier *et al.*, 1979). The baseline corrected water absorbance at the shorter wavelength (1450 nm) showed also similar non-linearity, but at higher water amounts. Earlier, it has been proposed that non-linearity of the output of a near-infrared moisture analyser, measuring at 1940 nm, could be used to estimate the optimal water amount for granulation (Miwa

et al., 2000). It is difficult to compare those results to the present ones, as the water amount ranges used are different, and because the restricted amount of sampling points in the present study does not allow observing subtle changes as in that work.

As discussed previously, it has been stated that the optimal liquid amount is located at the power consumption/torque plateau (Bier *et al.*, 1979). This plateau can however be rather wide, wherefore the torque measurement cannot be used alone to set the precise end-point. The slope of the spectrum baseline increased linearly in the water amount region where the impeller torque had a plateau (V). The SEM images indicated that the agglomerates changed during this period (V). If this is the case, the impeller torque fails to display changes taking place during the plateau stage, whereas changes are observed in the slope of the spectrum baseline. The slope increases due to changes in the path length of the NIR radiation. The main factors responsible for this change are the particle size and the consolidation of the powder mass. Additional studies, investigating relevant agglomerate properties (e.g. particle size distribution, flowability, compressibility and dissolution), are needed to clarify which method displays the agglomerate properties most accurately.

The slope of the spectrum baseline, the NIR water absorbance or the impeller torque do not display a single property of the agglomerates. Therefore, it can be difficult to determine what a change in these signals means in terms of a single property of the granules. This may be scientifically unsatisfactory, but however, these signals may give a process signature defining the progress of an optimal/satisfactory process. Nevertheless, despite the NIR displays many different granule properties, NIR spectroscopy has been applied earlier for non-invasive process monitoring of fluid bed granulation. NIR spectra were successfully correlated with moisture content (Frake *et al.*, 1997; Goebel and Steffens, 1998; Rantanen *et al.*, 1998, 2001) and/or particle size (Frake *et al.*, 1997; Goebel and Steffens, 1998). The fluid bed process, however, deviates from the high-shear granulation process in some aspects, which can also have an impact on the NIR monitoring. The extent of compaction is different in these processes; the high-shear process usually densifies the granules in a greater extent. Thus, the correlation between particle size and the NIR output may be less linear in a high-shear process, due to the effect of powder density on the NIR output. In addition, the movement of the particles in the process vessel is different in the two process

equipment. This might affect the effective sampling of the NIR probe, if in-line process monitoring would be applied.

5.2.2 Effect of hydrate formation on impeller torque

A slight decrease was observed in the beginning of the impeller torque curve of anhydrous theophylline, which was not perceived in that of theophylline monohydrate (V). This decrease disables the use of peak detection in the end-point control of the water addition phase. No such disturbance was observed in the anhydrous theophylline – MCC (1:1, w/w) and anhydrous theophylline – lactose monohydrate – MCC (1:1:1, w/w) mixtures (V). This might have been because the presence of water absorbing MCC delays the hydrate formation (IV). Therefore, it may be possible to use impeller torque measurements in process control, if the amount of hydrate forming API is small in the formulation and it contains water absorbing excipients.

5.3 Projections of wet granulation processes

The amount of data obtained by timely measurements from a process, especially when using spectroscopic methods, is large and the data is often multidimensional. In addition, the relation of the different factors affecting the process is often complex, as in wet granulation. Visualisation is an efficient way to present data, as can be seen in the scientific literature where plotting data is common. Multivariate data is however difficult to grasp by this method, because the highest amount of dimensions that can be used is three. Projection methods may be applied to compress large data sets to a few new features and thereby enabling the visualization of the data (Daszykowski *et al.*, 2003). In this chapter, principal components analysis (PCA) is used to visualize hydrate formation during wet granulation. In addition, principal components analysis is compared to self-organizing map (SOM) in the visualisation of a wet granulation process.

5.3.1 Principal components analysis in visualising an anhydrous to hydrate transition

The at-line NIR measurements of the wet masses of theophylline (II) were represented by three principal components explaining 99.9% of the variation in the data. These components created a 3-dimensional space, where the scores depicted a path. The

corresponding loadings showed that the first two principal components represented hydrate formation, observed as a OH-hydrate water band (1970 nm) and changes in CH vibrations (1600-1700 nm). The third principal component was affected by free water absorbance (1905 nm). Thus, the hydrate formation and the increase in free water may be monitored using NIR and PCA.

In addition to merely visualizing events taking place during a process, PCA can be applied for process monitoring and control. Earlier, PCA has been applied to process monitoring in other fields (Kourti and Macgregor, 1995; Wise and Kowalski, 1995) and recently, to process monitoring of a pharmaceutical synthesis (Svensson *et al.*, 2000). In addition, PCA has been proposed for monitoring the opposite event, dehydration, taking place during drying of API (Zhou *et al.*, 2003). PCA can be used to create a process signature, and is sensitive to all variation in the data, including also variations which may not have an influence on the product quality. If more focus on product variables is desired, PLS may be a better choice, as it takes in account those process variables that have an impact on the product variables (Nomikos and Macgregor, 1995).

5.3.2 Comparison of principal components analysis and self-organizing map

Despite of the potential of the spectroscopic methods, the traditional process monitoring techniques should not be forgotten but combined with the others by multivariate tools. In this work, this was conducted creating a process vector where all the different information available was included (VI). These vectors were then visualised using two different dimension reduction techniques. None of the measurement techniques were able to describe the state of the process alone at all stages (VI). The visualisation methods applied were however able to discriminate the different states of the process from each others.

The principal components analysis (PCA) provided a three-dimensional picture of the process (VI). However, all the principal components were influenced by almost all of the individual variables, making the interpretation of movement along a single principal component using of the loadings plot difficult. The self-organizing map (SOM) enabled the presentation of data on a two dimensional plane on which the progress of the process was depicted (VI). The SOM was not superior to the PCA but had some advantages. It could be used to investigate the relationships of the variables in

a simple manner using the component plots. These plots allowed the study of the original variables during the process and revealed non-linearities and global and local correlations of the variables. The SOM locates close data points in the same SOM element, whereas PCA conserves the minute differences in the data structure. In addition to merely studying a process and the relationships of different variables, the SOM have also been proposed for real-time process monitoring (Simula and Kangas, 1995). Examples have been presented from the chemical (Tryba and Goser, 1991) and metallurgical (Jämsa-Jounela *et al.*, 2003) industries.

6 CONCLUSIONS

Near-infrared (NIR) and Raman spectroscopy could be used to study hydrate formation in wet masses. The presence of two common excipients did not disable the measurements. It was observed that silicified microcrystalline cellulose retarded the hydrate formation of theophylline.

The NIR outputs showed changes at the impeller torque plateau i.e. at the optimal water amount. When granulating substances that require large liquid amounts, the slope of the baseline corrected water absorbance declined at the impeller torque plateau. In addition, the slope of the spectrum baseline increased at the plateau indicating that granule growth and consolidation took place also in this phase. The results suggested that NIR spectroscopy is applicable to process end-point monitoring of wet granulation in high-shear mixers.

The combination of process data made it possible to follow the wet granulation process in a manner which none of the individual process measurements enabled. Principal components analysis (PCA) and self-organizing maps (SOM) provided a way to visualise the progress of the process. The PCA reflected minute differences in the data whereas the SOM provided a more generalised picture. The study of the effect of the original variables to the state of the process was more straightforward using the SOM. When using only NIR data, the PCA was able to differentiate between hydrate formation and the increase of free water in the system.

The spectroscopic methods studied were able to give information of a solid-state transformation in wet masses and NIR reflected changes taking place during wet granulation. These methods could be used to elucidate physical changes induced due to wet granulation deliberately or unintentionally, such as increase in size and consolidation of agglomerates or processing-induced transformations. This kind of data, combined with other process data and analysed by dimension reduction tools can supply picture of the state of the process. Thus, the methods can be used to provide information which may contribute in creation of better process understanding. These methods can also be applied in real-time, enabling the development of process control tools that monitor several aspects of the process at the same time.

7 PERSPECTIVES

The results presented in this thesis showed that various kinds of information can be derived from wet granules by spectroscopic methods. In the future, it should be investigated, if the NIR and Raman methods presented in this thesis can be performed by non-invasive measurements delivering real-time data. The off- and at-line measurements used in the thesis are not fast enough to allow the gathering of real-time or near real-time data of the process, because granulation processes, especially high-shear granulation, are relatively fast. The creation of a non-invasive process interface is a science of its own, where different aspects, such as movement of the material in the process vessel, material and placement of the interface as well as temperature changes during the process, have to be considered. Successful non-invasive NIR measurements have been performed from fluid bed granulators, but there has not been published any non-invasive NIR measurements from high-shear mixers to date.

From a process analytical technology point of view, it would be desirable to measure and control *all* different aspects of a process that have an effect on the product performance. If real-time measurement of these would be possible, the self-organizing map would allow the monitoring and control of the desired process state. The state of the process could be displayed by a cursor indicating the node which represents each process state on the SOM. If fault situations and undesired process runs are introduced in the training of the SOM, it could also be used for fault diagnostics.

In the papers V and VI of this thesis, the focus was on the water addition phase of high-shear granulation. In the future, it would be interesting to investigate if NIR spectroscopy could be use to monitor the wet massing phase as well. At the moment, the wet massing phase is usually controlled by running the process a certain period of time determined by experiments. The slope of the NIR spectrum baseline might permit a more flexible process control than the present way.

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