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Polymorph screening of a former drug candidate

Ph.D. Thesis

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LIST OF ORIGINAL PUBLICATIONS

- I.** Szűts, A.; **Láng, P.**; Ambrus, R.; Kiss, L.; Deli, M. A.; Szabó-Révész, P.:
Applicability of sucrose laurate as surfactant in solid dispersions prepared by melt
technology
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- II.** **Láng, P.**; Kiss, V.; Ambrus, R.; Farkas, G.; Szabó-Révész, P.; Aigner, Z.; Várkonyi,
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- III.** **Láng, P.**; Várkonyi, E.; Ulrich, J.; Szabó-Révész, P.; Aigner, Z.:
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ABSTRACTS

- IV. Láng, P.;** Kiss, V.; Ambrus, R.; Farkas, G.; Szabó-Révész, P.; Aigner, Z.; Várkonyi, E., Screening of polymorph forms of a former drug candidate:
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- V. Láng, P.;** Kiss, V.; Szabó-Révész, P.; Aigner, Z., Várkonyi, E., Potenciális farmakon-jelölt polimorfia szűrése:
MKE Kristályosítási és Gyógyszerformulálási Szakosztály Kerekasztal Konferenciája, Balatonszemes, 26-28. October 2012.
- VI. Aigner, Z., Láng, P.;** Szabó-Révész, P.; Várkonyi, E.:
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- VII. Láng, P.;** Várkonyi, E.; Szabó-Révész, P.; Aigner, Z.:
Relative stability of a former-drug candidate's polymorphs, 9th Word Meeting on Pharmaceutics, Biopharmaceutics and Pharmaceutical Technology, Lisbon, Portugal, 31 March-3 April 2014., Starting materials /P-254/
- VIII. Láng, P.;** Várkonyi, E.; Ulrich, J.; Szabó-Révész, P.; Aigner, Z.:
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ABBREVIATIONS

API	Active pharmaceutical ingredient
DSC	Differential scanning calorimetry
DTG	Differential thermogravimetry
CPS	Counts per second
EDS	Energy dispersive spectroscopy
EMA	European Medicines Agency
FDA	Food and Drug Administration
HLB	Hydrophilic-lipophilic balance
HSM	Hot-stage microscopy
HTS	High-throughput screening
ICH	International Conference on Harmonisation
IR	Infrared
MTDSC	Modulated-temperature differential scanning calorimetry
o/w	Oil in water
RH	Relative humidity
SE	Sugar ester
SEM	Scanning electron microscopy
ssNMR	Solid-state nuclear magnetic resonance
TG	Thermogravimetry
TG-IR	Thermogravimetry coupled with infrared spectroscopy
TG-MS	Thermogravimetry coupled with mass spectroscopy
VH-XRPD	Variable humidity X-ray powder diffractometry
VT-XRPD	Variable temperature X-ray powder diffractometry
w/o	Water in oil
XRPD	X-ray powder diffractometry

1. INTRODUCTION

Investigations of the polymorphism of APIs are currently essential in pharmaceutical research and production. This process is known as "polymorph screening". The polymorph screening of organic materials is an extremely complex and multifaceted field, which poses considerable challenges for innovators and generic companies from the aspects of both pharmaceutical and intellectual property rights.

Polymorphism is a common phenomenon among APIs. Some literature sources suggest that it occurs in 32-51% of solid materials [1], whereas other surveys indicate that ~ 90% of crystalline active ingredients have polymorphs [2]. The physico-chemical properties of polymorph modifications such as melting point, hardness, solubility, dissolution rate, etc. can differ as a consequence of the different crystal structures and this may influence the formulation, the storage, the absorption, the toxicity, the efficiency and finally the bioavailability of the API. The importance of polymorph screening is outstanding since the drug registration authorities require a precise description of the characteristics of a solid-state API and a drug candidate can be placed on the market only in the case of a specific polymorph for which long and expensive biological, toxicological and clinical studies have been carried out [3-5]. The intended use of a different polymorph of the same API demands the repetition of these studies, which involves a loss of time and money. The recommendations of the ICH, prompted by the requirements of the authorities, regulate the authorisation of original and generic products, this being accepted by the FDA and the EMA. In the synthetic process, most APIs and their salts are purified and isolated by crystallization from an appropriate solvent [6]. Many factors can influence crystal nucleation and growth during this process, including the composition of the crystallization medium (solvent, solvent combinations, additive type, etc.) and the process(es) used [7-9]. Since pharmaceutical compounds often have a great number of solid phases, even in a metastable state, their interpretation is difficult because of kinetic factors. Various techniques are therefore used for the study of polymorphism [10].

Pharmaceutical dosage forms contain both active ingredients and excipients. Although excipients must be inert, they may influence the absorption and bioavailability of the drug in such a way as to increase the stability of the formulation, to facilitate the liberation of the API, etc. [11]. Additives and impurities can influence the kinetic stability of a polymorph in a solution or a suspension by affecting both the nucleation and the growth rate [12, 13]. Their

presence may promote the formation of a metastable polymorph, which may otherwise rapidly transform to a more stable polymorph [12, 14]. Polymorph screening that is performed with additives may therefore lead to different results from a screening performed with a pure batch [13]. Among the additives currently utilized in the pharmaceutical industry are the SEs, which have likewise long been preferred in the food and cosmetic industries too. They can be applied as emulsifiers, solubility and dissolution rate enhancers, gel-formers and crystal growth enhancers or inhibitors [15-21]. *Szűts et al.* reported that a certain type of SE influenced the degree of crystallinity of a model drug [22]. Accordingly, since SEs can affect the crystal structure of the API, it is useful to investigate the structural changes during crystallization processes when SEs are used as additives.

2. AIMS

Polymorph screening is a very complex process during preformulation research. It is not sufficient to investigate merely the effects of the organic solvents and the temperature. The presence of the different additives, such as the SEs, must also be considered. These surfactants may cause changes in the polymorphic form during crystallization, and these may finally affect the bioavailability of the drug.

The aim of my PhD work was therefore to develop a polymorph screening method on a model drug and to investigate the influence of different SEs during the crystallization process.

- A. The main aim of my investigations was to develop a polymorph screening method on a failed drug candidate. Different crystallization and transformation methods were used to generate the polymorphs of the model drug. Eight polymorphs were obtained and were characterized by light microscopy, SEM, XRPD, DSC and Raman spectroscopy. A special dissolution test was developed with which the eight polymorphs could be well distinguished.
- B. After the characterization of the different polymorphs, their relative stabilities were investigated by means of a slurry conversion method, VH-XRPD, VT-XRPD and DSC.
- C. Finally, different types of SEs (widely used as additives at present) were studied during the crystallization of the polymorphs in order to establish their polymorph-changing effects.

3. LITERATURE SURVEY

3.1. Polymorphism

The idea of polymorphism was introduced by *Mitscherlich* at the beginning of the 19th century during his investigations of arsenates and phosphates [23]. Polymorphism is the ability of a compound in the solid state to exist in different crystalline forms with the same chemical composition. The phenomenon of a molecule existing in more than one solid-state structure is a result of differences in packing arrangement and/or molecular conformation [8]. ICH Q6A defines polymorphism as “some new drug substances exist in different crystalline forms which differ in their physical properties. Polymorphism may also include solvation or hydration products (also known as solvatomorphism) and amorphous forms” [24].

The polymorphism of a chemical element is known as allotropism. As a simple example, diamond and graphite are two allotropes of carbon. Another example is that of phosphorous (P₄) in cubic (white), monoclinic (purple) and orthorhombic (black) forms [25].

Mention should also be of an interesting phenomenon related to polymorphism: "disappearing polymorphism" [26-28]. This refers to a situation where a previously prepared form no longer appears after a more stable form is obtained [29]. The numerous examples of disappearing polymorphism include benzylidene-dl-piperitone, benzocaine picrate, mannose, etc. [26].

3.2. Thermodynamic relations in polymorphs

In terms of thermodynamic relations, polymorphic modifications may be *monotropes* or *enantiotropes* [1], depending on their stability with respect to temperature and pressure. If one of the polymorphs is stable (i.e. it has a lower free energy content and solubility) over certain ranges of temperature and pressure, while the other polymorph is stable over different ranges of temperature and pressure, the two polymorphs are said to be enantiotropes. On the other hand, it may occur that only one polymorph is stable at all temperatures below the melting point, all the other polymorphs being unstable. Such polymorphs are said to be monotropes (Fig. 1).

Fig. 1 Variation of energy with temperature for enantiotropic and monotropic systems. Curves HA, HB and HL relate to enthalpy, $\Delta H_{f,A}$ and $\Delta H_{f,B}$ to enthalpy of fusion, and GA, GB and GL to free energy. T_t is the transition temperature, M.P. is the melting point and ΔH_t is the enthalpy of transformation [25]

In an enantiotropic system, a reversible transition is observed at a definite transition temperature at which the free energy curves intersect before the melting point is reached. By contrast, for a monotropic system, the free energy curves do not intersect, and therefore no reversible transition is observed below the melting point. The polymorph that demonstrates the higher free energy curve and higher solubility is called an unstable polymorph. It is generally possible to distinguish monotropes and enantiotropes through their heats of fusion. Endothermic polymorphic transitions indicate that enantiotropes are involved, whereas exothermic ones point to monotropes [25].

3.3. The importance of polymorphism in pharmaceuticals

The different polymorphs exhibit different physical and physico-chemical properties, such as habit, colour, density, melting point, solubility, dissolution rate, etc. [30]. One reason for the importance of polymorphism is that these differences may affect the pharmaceutical processing, the bioavailability, the toxicity and the stability of the drug product, and hence the therapeutic efficacy of the API [31]. Another aspect is the possibility of a patent. Two of the requirements for a patent are *novelty* and *non-obviousness*. By definition, a new crystal form is novel. Since crystal forms cannot be predicted *a priori*, they are not obvious. Many new

crystal forms are therefore patentable and have been patented [32]. As most APIs in the pharmaceutical field are formulated and marketed in crystalline form, this explains the importance of this topic.

3.4. Solid form screening

Solid form screening is a standard procedure in drug development, but it was not until the late 20th century that the pharmaceutical industry overall became aware of polymorphism, even though the phenomenon had been known since the early 19th century [33]. It was not until a number of production problems appeared, such as the sudden occurrence of another form of ritonavir (with low solubility and bioavailability) [34], that the industry started to take polymorphism seriously. How extensive polymorph screening should be during the early development process is well illustrated by *McCrone*, who in 1965 wrote as follows: "It is at least this author's opinion that every compound has different polymorphic forms, and that, in general, the number of forms known for that compound is proportional to the time and money spent in research on that compound" [35]. The aim of solid form screening is to find the optimum polymorph with the best characteristics (including the physico-chemical, pharmaceutical and biopharmaceutical properties) for development. In general, the most stable form is preferred over other forms because of its lower tendency to solid phase transformations [36]. However, metastable forms are sometimes deliberately chosen, usually for better solubility and therefore bioavailability [37]. Solid form screening can be carried out both experimentally and computationally. Experimental screening consists in the preparation step, during which various forms are generated and isolated, and the analysis step, which involves the use of various measurement techniques and analysis of the data. Computational methods of polymorph prediction have evolved markedly during recent years, though they cannot be fully relied on yet.

HTS has been integrated into the pharmaceutical discovery process. It utilizes fully automated robotic systems capable of performing thousands of crystallizations per week with the consumption of only a few grams of API [38, 39]. It is most commonly carried out in 96-well plate systems in which the particular solid (~ 0.5-10 mg/sample), dissolved in a suitable solvent, is initially dispensed by using automated liquid handling systems. Different levels of supersaturation can be achieved, for example by heating/cooling and evaporation, and by varying the nominal concentration of the API. During a polymorph screening process, the interplay between kinetic and thermodynamic factors must be extensively utilized to discover all the relevant solid forms of a compound in question [36].

Thus, the key experimental technique used to perform solid form screens is crystallization. The methods applied to generate polymorphs are summarized in **Table 1**. The techniques that I used are discussed in detail.

Table 1 Methods of generating polymorphs [29]

Crystallization from a single or mixed solvents/HTS [40-44]
Thermal activation of solid substrates [45]
Crystallization from the melt [46-48]
Desolvation/dehydration of solvates/hydrates by heat or by reslurrying [49, 50]
Crystallization in nano-confined structures [51-53]
Seeding/pseudoseeding [54, 55]
Solution-mediated polymorphic transformation/slurry conversion methods [56-59]
Solid-state polymorphic transformation [49, 60]
Mechanical activation of a solid substance [61, 62]
Crystallization in a capillary tube [63, 64]
Exposure to vapour at high or low humidity [65-67]
Exposure to organic vapour [68]
Direct crystallization on molecular substrates [69-74]
Crystallization in the presence of tailor-made additives [75-89]
Laser-induced crystallization [90, 91]
Crystallization from a supercritical fluid [92-94]
Structure prediction <i>in silico</i> [95-99]

Crystallization from single or mixed solvents

Polymorph screening is generally carried out by crystallization from single or mixed solvents, through cooling or evaporation of the solvent or addition of an antisolvent to the solution [100]. It is a great challenge to select appropriate solvents for solid screening: it is necessary to consider physicochemical properties such as the hydrogen-bond acceptor/donor propensity, polarity/dipolarity, dipole moment, dielectric constant, miscibility, etc. [101]. Classically, the crystallization process is described in terms of two distinct steps, *nucleation* and *crystal growth* [102], with the resulting physical form being a consequence of the kinetic relationship between these two elementary processes. Many factors (e.g. heating and cooling rates, temperature, evaporation rate, degree of supersaturation, pH or pH change of the medium, etc.) can influence the crystallization process, and polymorphs are therefore formed [29].

Thermal transformation

As concerns the thermodynamic relationship between polymorphs, it was stated in section 3.2 that any given two polymorphs can be either monotropic or enantiotropic [29]. In an enantiotropic system, the form that is metastable at room temperature can be obtained by heating the form that is stable above the transition temperature. In a monotropic system, the stable form at room temperature can be obtained by heating the metastable form at any temperature. The rate of transformation can be facilitated by heating the metastable form at high temperature. By starting with the stable form, it is impossible to obtain the metastable form by thermal activation in a monotropic system. The transition temperature can be estimated by observing the transition events during DSC measurements [103], by slurrying mixtures of polymorphs over certain temperatures [104, 105] or by calculating the Gibbs free energy differences between polymorphs over temperature ranges via direct heat capacity measurements [106].

Desolvation/dehydration of solvates/hydrates by heating or by reslurrying

On increase of the number of experiments during the screening process through extension of the HTS and polymorphic screening methods, the number of hydrates/solvates discovered during the screening process may increase [107]. When dehydration or desolvation occurs, such solvates/hydrates can undergo phase transitions which may result in non-solvated/anhydrous polymorphs [108, 109] or in the loss of crystallinity and the formation of amorphous products [110]. Isomorphic desolvates/dehydrates can sometimes be formed, which means that solvent/water molecules leave the crystals without affecting the crystal structures of the solvates/hydrates [111, 112]. Desolvation/dehydration processes are usually conducted at low relative humidity or low organic vapour pressure. It has recently been demonstrated that desolvation with the use of the reslurrying of solvates in a solvent with poor solubility at low or high temperature can yield pharmaceutically relevant polymorphs [113].

3.5. Additive-induced polymorph selection

Additive-induced polymorphism has always interested solid-state chemists for fundamental and practical reasons [114]. Additives can inhibit nucleation by being incorporated into the prenuclear aggregates/crystals or by binding to the nuclei/crystals. There are three possibilities for the inhibition of nucleation/crystal growth by means of additives:

As the possibility additives prevent prenuclear aggregates of a particular form from growing into a nucleus with a critical radius by creating defects in the aggregates [115]. As a result, the prenuclei redissolve into the solution. In this respect, additives can decrease the rate of nucleation by increasing the critical supersaturation for nucleation and/or interfacial tension. Alternatively, additives can favourably attach to the prenuclei of a certain form of a polymorph or enantiomer [116], other polymorphs remaining unaffected by the additives. As a consequence, the unaffected prenuclei grow into the nuclei with a critical radius size, and become the resultant crystals.

The third possibility is for additives to attach to the fastest-growing face of the stable polymorph, which prevents the stable form from growing [117]. As a result, the metastable polymorph crystallizes [29]. Inhibition of the growth of a specific face will result in changes in morphology, dissolution rate and/or polymorph selection [118]. Different sucrose oligoesters added to palm kernel oil have been described as crystallization-accelerator additives [119].

3.6. The importance of sucrose esters in pharmaceuticals

SEs are widely used in the food and cosmetics industries, and there has recently been great interest in their applicability in different pharmaceutical fields [120]. They are biodegradable, natural, non-ionic surface-active agents consisting of sucrose as hydrophilic moiety and fatty acids as lipophilic groups (Fig. 2). SEs can be characterized by their HLB values. Thanks to the wide range of these values (1-16), the SEs can be applied in many fields of pharmaceutical technology, such as the release or the absorption of modifying agents, o/w or w/o emulsifiers, wetting and solubilizing agents or lubricants [121-130].

Fig. 2 General chemical structure of SEs

Table 2 Examples of the different types of SEs

Type	Fatty acid	HLB
B 370	Behenic acid	3
S 370	Stearic acid	3
S 1570	Stearic acid	15
O 1570	Oleic acid	15
P 1670	Palmitic acid	16
M 1695	Myristic acid	16
L 595	Lauric acid	5
L 1695	Lauric acid	16

3.7. Methods of investigation polymorphism and solvatomorphism

Characterization by different microscopic methods

A complement of physical characterization methods have been developed for the study of polymorphs and solvates, including microscopy, crystallography and thermal analysis.

Optical crystallography, which includes **light microscopy**, is directly applicable to organic chemicals. This technique is based on the fact that light travels at different speeds in different directions in a crystal [131]. During the examination of crystals by microscopy, their 3D shapes can be characterized simply and quickly. Light microscopy can also be used to estimate the particle size distribution in a powder sample. A quicker and more efficient method is to use a computer-controlled image analyser with a motorized specimen stage and a camera [1]. Another combination of traditional microscopy with a hot-stage accessory is **HSM**. During pharmaceutical development, HSM is a preferred choice that is mostly used to support DSC results, because it provides visual and semiquantitative information concerning the transition of pharmaceutical polymorphs [132]. It provides a unique insight into polymorphic transitions and the thermal behaviour exhibited by different crystal forms of a compound [133, 134]. **SEM** has many advantages over traditional microscopy. It is used to observe the surfaces of materials at high resolution [135]. Depending on the instrument used, specimens can be magnified roughly between 10 and 100,000 times, thereby allowing the detection of much greater detail than with light microscopy [136, 137]. **EDS** is a powerful technique that is ideal for revealing what elements are present in a particular specimen. The initial EDS analysis usually involves the generation of an X-ray spectrum from the entire area

scanned by SEM. Through the process of X-ray mapping, information about the elemental composition of a sample can then be overlaid on the magnified image of the sample [138].

Different spectroscopic methods

During polymorphism screening, additional valuable information can be obtained by vibrational spectroscopy methods such as **Raman** and **IR** spectroscopy, which are most widely used since they are easy to handle and involve relatively low cost. Conventional IR spectrometers have a low energy cut-off around 200-400 cm^{-1} , while Raman scattering is the only method with which the complete vibrational spectrum of a molecule (from 4 to 4000 cm^{-1}) can be easily recorded. Raman spectroscopy generally has better spectral selectivity (for the distinction of polymorphic forms including the amorphous state) as the symmetric stretching linked to the carbon skeleton is more expressed than in IR spectroscopy, and in contrast with IR spectroscopy, it is not disturbed by the presence of water. The main advantages of Raman and IR spectroscopy include the fast and non-invasive measurements, the *in situ* and *on-line* monitoring capabilities, the lack of sample preparation, quantitative analyses, etc. [32, 139, 140].

SsNMR spectroscopy with cross-polarization and magic-angle spinning is a powerful crystallographic method for the investigation of crystalline polymorphs and the relative crystalline and amorphous contents of pharmaceutical excipients. It can also be used to identify the number of crystallographically non-equivalent sites, since each crystallographically unique molecule in the unit cell gives rise to an NMR signal [141].

X-ray methods

XRPD is the front-line technique for the analysis of polymorphs. It can be used (like IR spectroscopy) for the quantitative analysis of polymorph mixtures, and as an analytical tool for high-throughput screening with some modifications. However, when the reference powder patterns of pure forms are not available, it is often difficult to ascertain whether a powder pattern is from the mixture or pure material. In that case, single-crystal X-ray analysis is necessary [29]. XRPD is commonly used to investigate the structures of variable hydrates, which are crystalline species that contain non-stoichiometric amounts of water held within channels in the crystal lattice. **VH-XRPD** studies with XRPD instruments equipped with a variable-humidity sample stage allow *in situ* analyses of samples externally conditioned under various relative humidity environments [142]. By means of **VT-XRPD**, a technique in which

XRPD experiments are carried out at different temperatures [143], complex pharmaceutical solid-state reactions, including crystal structure transformations, can be characterized *in situ* [144, 145]. The VT and VH sample chambers of the XRPD instrument allow the crystal form changes associated with the changing conditions to be followed [146]. **Single-crystal X-ray** analysis is a non-destructive technique which provides information on the internal lattices of crystalline substances, and also unit cell parameters such as bond lengths, bond angles and unit cell types [147]. However, it is often very difficult to obtain a crystal suitable for single-crystal X-ray analysis, and especially metastable forms.

Thermal analysis

Other important methods that are widely used in the pharmaceutical industry for the characterization of polymorphism, solvation and purity are the various forms of thermal analysis, which include **TG**, **DTG** and **DSC**. Crystalline states or forms exhibit different levels of thermodynamic stability and an unstable form can melt at a temperature significantly lower than the melting point of the thermodynamically stable form. The different polymorphic forms can therefore be identified through their melting profiles and melting points, which can be detected by calorimetric methods. The samples are subjected to increasing temperature in order to detect peaks reflecting the loss of matter (DTG), or the release of energy in the form of heat (DSC). **DSC** measures heat flow into or from a sample during heating or cooling or under isothermal conditions. For the characterization of solvates and hydrates, the use of both **DSC** and **TG** is needed [148]. Although these two techniques are quite sensitive, they are not specific: the solid transformations may have too low energies, impurities have a high impact on the melting points and the amorphous content influences the measured melting enthalpies [10, 149]. DSC data can be also used to determine the relative stability of polymorphs [150]. Combined techniques can be extremely helpful for both the characterization and the determination of the sequence of stability of polymorphs. **TG-IR** or **TG-MS** is used if the identity of the volatile component is determined *in situ* [10]. **HSM** combines the best properties of microscopy and thermal analysis to permit the solid-state characterization of materials as a function of temperature [151]. **VT-XRPD** provides the required information to ascertain whether an evolved gas is due to solvent incorporated in the crystal lattice or physically adsorbed on the solid [152].

Other methods

For thermodynamic reasons, a drug exhibiting crystal polymorphism should display different thermodynamic activities, depending upon its crystalline modification. The rate of the release of the drug from any solid dosage form, *in vivo* or *in vitro*, may be strongly dependent on this when the rate process is controlled by diffusion. Since solubility or drug dissolution is related to drug absorption, it is also very important to perform dissolution studies. During the initial stages of the development of a pharmaceutical, the polymorphic form should be selected that best meets the two most important requirements of stability and solubility [153]. The solubility of a crystal in a solvent is a reflection of how stable the crystal is. The crystalline structure/polymorphic form with the highest stability always has the lowest solubility. The more stable it is, the lower its tendency to dissolve in the surrounding solvent [154].

Methods of relative stability examination

During pharmaceutical production, materials pass through many processes which may cause polymorph transformation, such as compression, granulation, grinding, drying or pulverization [143, 155]. Polymorphs have different lattice energies, and forms with higher energy tend to transform into forms with lower energy [8]. Storage conditions, such as humidity or temperature, affect the stability of crystal forms [156], and clarification of the thermodynamic stability relations between the polymorphs is therefore essential during the polymorph screening process. The most stable polymorph usually possesses the lowest Gibbs free energy [157]. The **slurry conversion method** is a technique in which an experiment is performed with a programmable temperature control hot stage in which the sample is stirred in suspension form at different temperatures. After a certain number of hours or days, the excess solid is removed for analysis by XRPD [158]. *In situ* crystal structure transformations can readily be followed by **VH-XRPD** and **VT-XRPD** [144-146]. Other important methods of detecting polymorph transitions are calorimetric methods such as **DSC** or **MTDSC**. **MTDSC** differs from conventional DSC in that the sample is subjected to a more complex heating program, incorporating a sinusoidal temperature modulation accompanied by an underlying linear heating jump ramp. While DSC measures the total heat flow, MTDSC can also distinguish reversible (heat capacity component) and non-reversible (kinetic component) heat flows [159]. The heating rate may have a great influence on the kinetics and the resolution of peaks in DSC curves [154]. At a comparatively high heating rate (e.g. 100 °C min⁻¹), the kinetics of the melting transition is changed as if there were not enough time for

recrystallization of the higher melting form [160]. With **fast-scan DSC**, the melting of the metastable polymorph can be distinguished from any subsequent recrystallization because the later event is moved to a higher temperature and provides separation of the events [161].

4. MATERIALS AND METHODS

4.1. Materials

My Ph.D. work related to the polymorphism of a failed drug candidate, 3-[2-([1-(2-cyclohexylethyl)-5-(2,5-dimethoxy-4-methylphenyl)-1H-1,2,4-triazol-3-yl]amino)carbonyl]-6-methoxy-4,5-dimethyl-1H-indol-1-yl]propanoic acid (Sanofi Pharmaceutical Company, Budapest, Hungary) (Fig. 3).

Fig. 3 Chemical structure of the model drug

In the first step relating to the crystallization of the various polymorphic forms of the model drug, I used different organic solvents of analytical grade: acetonitrile, methanol, 96% ethanol, abs. ethanol, isopropanol, acetone, 2-butanone, toluene (Merck, Budapest, Hungary), dichloromethane, ethyl acetate, butyl acetate, chloroform, 1,4-dioxane (Reanal, Budapest, Hungary) and tetrahydrofuran (Aldrich, Budapest, Hungary).

After crystallization of the model drug in the pure solvents, the same method was repeated in the presence of SEs (Mitsubishi-Kagaku Foods Corporation, Japan) with low (S 370 and L 595) or high HLB values (S 1570, O 1570 and D 1216).

4.2. Sample preparation

Preparation of polymorphs by crystallization

Different techniques were used for the preparation of the polymorphs (**Table 3**). **Crystallization by shock cooling:** ~100 mg of the raw material was dissolved at the boiling point of the solvent to give a saturated solution which was then diluted with a small amount of the solvent. The hot solution was filtered by a "Canula 10 µm" filter into a vial immersed into crashed ice.

Crystallization by slow cooling: The same process of dissolution was used, but the filtered, hot solution was cooled down slowly (-3 °C/h).

Crystallization by slow evaporation: The products were the dried residues of the clear filtrates of the samples prepared by shock cooling.

Preparation of polymorphs by heating transformation

Two of the former drug candidate's polymorphs (Form II and IVb) could be generated by heating processes at various temperatures for different periods of time from the polymorphs generated by crystallization. When Form I was heated on 160 °C during 6 hours, it transformed to Form II. In case of Form IVb the same process was applied on Form IVa, but on higher temperature (205 °C). (At the early beginning of the polymorph screening of this former drug candidate, it was not possible to decide if Form IVa and IVb are a mixture of two polymorphs or they are pure polymorphs, but the further analytical investigations could prove that they are separate forms.)

Preparation of polymorphs by crystallization in the presence of sucrose esters

After the differentiation of the polymorphic forms by means of analytical examinations, the single-solvent recrystallizations were repeated in the presence of one or other SE. The different SEs were chosen on the basis of their HLB values (**Table 4**). The solubilities of these five SEs in the organic solvents used were determined previously during the single-solvent recrystallizations. During the additive-induced crystallization, the given SE was simultaneously dissolved in the given solvent with the model drug in a mass ratio of 1:1.

Table 3 The techniques used for the preparation of the polymorphs

Form	Method	Solvent	Circumstances
I	crystallization	96 %, abs. ethanol	shock cooling
II	heating	-	160 °C/6 h (from Form I)
III	crystallization	isopropanol, 2-butanone, butylacetate, ethylacetate, toluene	shock cooling
IVa	crystallization	methanol	shock cooling
IVb	heating	-	205 °C/6 h (from Form IVa)
V	crystallization	chloroform	shock cooling, slow cooling, slow evaporation
VI	crystallization	acetone, methanol, dichlormethane, 1,4-dioxane	shock cooling, slow cooling, slow evaporation
VII	crystallization	acetonitrile	shock cooling

Table 4 SEs and solvents used during the additive-induced crystallizations

SE	Solvent	HLB	API:SE mass ratios
S 370	chloroform, dichloromethane	3	1:1
S 1570	chloroform	15	1:1
O 1570*	96%, abs. ethanol, butyl acetate, methanol, chloroform, dichloromethane, 2-butanone, isopropanol	15	1:1, 1:2, 1:4, 2:1, 4:1
L 595*	96%, abs. ethanol, butyl acetate, methanol, chloroform, dichloromethane, 2-butanone, isopropanol, ethylacetate, toluene, acetone	5	1:1, 1:2, 1:4, 2:1, 4:1 1:1
D 1216	96%, abs. ethanol, 2-butanone, isopropanol, methanol, chloroform, acetone	16	

* In the cases of the SEs O 1570 and L 595, which had polymorph-changing effects, and additional ratios were also tested.

4.3. Polymorph investigation methods

The particle size distribution of the drug candidate was investigated by **light microscopy** using a LEICA Image Processing and Analysis System (LEICA Q500MC, LEICA Cambridge Ltd., Cambridge, United Kingdom). The particles were described in terms of their length, breadth, surface area, perimeter and roundness which is a shape factor giving a minimum value of unity for a circle. This is calculated from the ratio of perimeter squared to area using the following formula:

$$Roundness = \frac{Perimeter^2}{4 \cdot \pi \cdot Area \cdot 1.064}$$

The adjustment factor of 1.064 corrects the perimeter for the effect of the corners produced by the digitization of the image.

The morphology of the particles was examined by **SEM** (Hitachi S4700, Hitachi Scientific Ltd., Tokyo, Japan) having a magnification of 1000x. A sputter coating apparatus (Bio-Rad SC 502, VG Microtech, Uckfield, United Kingdom) was applied to induce electric conductivity on the surface of the samples. The air pressure was 1.3-13.0 mPa.

Raman spectra were recorded at room temperature by using a Bruker SENTERRA Dispersive Raman Microscope (Bruker Optik GmbH, Ettlingen, Germany) equipped with Nd-YAG (532 nm) and diode (785 nm) excitation lasers and a cooled CCD detector. The system was fitted with a motorized XYZ sample stage. The equipment was controlled by OPUS 6.5 software (Bruker Optik GmbH, Ettlingen, Germany). Samples were analyzed on glass slides.

Following a parameter optimization process, the samples were measured by Nd-YAG (532 nm) laser at a laser power of 10 mW. An integration time of 5 s, and 3 scans were used for each measurement, and spectra were collected over the Raman shift range of 3500-60 cm^{-1} at a resolution of 3-5 cm^{-1} . The microscope magnification was 200 $\times$ and a certain crystal was selected for analysis with a 50 $\mu\text{m} \times 1000 \mu\text{m}$ grating. Single Raman spectra of the individual crystals were collected in each case.

XRPD spectra were recorded with a BRUKER D8 Advance diffractometer (Bruker AXS GmbH, Karlsruhe, Germany) system with Cu $K\alpha_1$ radiation ($\lambda = 1.5406 \text{ \AA}$) over the interval 2.5-40 $^\circ/2\theta$. The measurement conditions were as follows: target, Cu; filter, Ni; voltage, 40 kV; current, 40 mA; time constant, 0.1 s; angular step 0.016 $^\circ$. Detector: NaI (Tl) scintillation detector.

DSC curves were obtained by a Mettler Toledo DSC27HP apparatus (Mettler-Toledo AG, Greifensee, Switzerland) under the following conditions: sample weight: about 2-3 mg; sample holder: aluminum crucible (40 μL) with lid; nitrogen flow rate: 100-150 mL/min; heating rate: 10 $^\circ\text{C}/\text{min}$ from 25 $^\circ\text{C}$ up to 250 $^\circ\text{C}$. In every case, samples were held at 25 $^\circ\text{C}$ for 10 min before recording.

Dissolution rate was also studied by a modified paddle dissolution apparatus (Pharmatest, Hainburg, Germany) at a paddle speed of 100 rpm. 100 mL of dissolution medium was placed in a 37 $^\circ\text{C}$ ($\pm 0.5 \text{ }^\circ\text{C}$) bath and the examination was performed for 2 h. Because of the poor solubility of the model drug, neither gastric acid nor intestinal fluid could be used alone. Therefore, an appropriate dissolution medium had to be developed. The preliminary examination of the model compound showed that its solubility depends on pH: at low pH (gastric acid) is worse than at neutral pH (intestinal fluid), therefore intestinal fluid (pH = 6.8) was chosen, but the dissolution even in intestinal fluid was not up to 100 %. As all the 8 polymorphs dissolved well in 96 % ethanol, the optimum mixture of 96 % ethanol: intestinal fluid (pH = 6.8) was found of a volumetric ratio of 3:7. The concentration of each polymorph solution was determined spectrophotometrically at 324 nm (Unicam UV/vis spectrophotometer, Unicam Limited, Cambridge, United Kingdom). The dissolution experiments were conducted in triplicate and standard deviation was also calculated.

4.4 Relative stability examination of the polymorphs

Slurry conversion method

An automatic laboratory reactor system (Avantium Crystal 16, Amsterdam, The Netherlands) was used to investigate the relative stabilities of the different polymorphs in suspension. Crystal 16 is a multiple reactor system working as a parallel crystallizer on a 1 mL volume scale, equipped with an online turbidity probe. The organic solvents for the relative stability examination were chosen with regard to the solubility properties of each polymorph. The different polymorphs were stirring in suspension in 96 % ethanol and in silicone oil. 96 % ethanol was regarded as a "moderately good" solvent of the polymorphs and silicone oil as a heat-transferring medium, because in some cases the polymorph transformation is achieved at high temperature ($> 200\text{ }^{\circ}\text{C}$), therefore it had to be look for an inert, non-volatile liquid. 100 mg samples of the polymorphs were examined in 1 mL of 96 % ethanol or silicone oil for a maximum of 130 days at room temperature, 50 or 70 $^{\circ}\text{C}$ (in the case of ethanol) or at 200 $^{\circ}\text{C}$ (in case of silicone oil). Samples of the suspensions were taken out from time to time with a glass pipette, filtered and placed on a Si low-background smooth-surfaced sample holder, and the crystals obtained were measured by XRPD.

Variable humidity and temperature X-ray powder diffractometry

VH/VT-XRPD patterns were recorded with a Bruker D8 Advance diffractometer (Bruker AXS GmbH, Karlsruhe, Germany) system with Cu $\text{K}\alpha_1$ radiation ($\lambda = 1.5406\text{ \AA}$) in the interval $2.5\text{--}40\text{ }^{\circ}2\theta$. The diffractometer was equipped with a hot-humidity chamber (MRI Physikalische Geräte GmbH, Karlsruhe, Germany) controlled by an Ansyco Sycos H-Hot (Analytische Systeme und Komponenten GmbH, Karlsruhe, Germany) and a Vântec 1 line detector (Bruker AXS GmbH, Karlsruhe, Germany), which indicated the phase transitions of the polymorphs directly in the diffractometer chamber. The measurement conditions were as follows: target, Cu; filter, Ni; voltage, 40 kV; current, 40 mA; time constant, 0.1 s; angular step, 0.007° . The parameters of the VH-XRPD investigations: humidity between 20 and 80 RH% in 10 RH% increments, and repeated measurement at 20 RH%. The temperature was 30 $^{\circ}\text{C}$. The VT XRPD studies were carried out between 30 and 230 $^{\circ}\text{C}$, in increments of 5 $^{\circ}\text{C}$. After the series of measurements, the samples were cooled back to 30 $^{\circ}\text{C}$ and measurements were repeated immediately after cooling and one day later.

Differential scanning calorimetry

The DSC analysis was carried out with a Mettler Toledo STARe thermal analysis system, version 9.30 DSC 821e (Mettler-Toledo AG, Greifensee, Switzerland), at a linear heating rate of 1, 10 or 30 °C min⁻¹, with nitrogen as carrier gas (100 mL min⁻¹). The sample weight was in the range 2-5 mg and examinations were performed in the temperature interval 25-250 °C, in a sealed 40 µL aluminium crucible having two leaks in the lid. In every case, samples were held at 25 °C for 10 min before measurements. The MTDSC parameters were as follows: temperature interval, 25-250 °C; heating rate: 10 °C min⁻¹; amplitude: 0.5 or 1 °C; period: 0.5 or 1 min.

5. RESULTS

5.1. Characterization of the polymorphs

The objectives here were to distinguish the different polymorphic forms by analytical methods, to investigate the relative stability of the polymorphs and to determine the results of SE-induced polymorph selection.

Light microscopy

First, the particle size distribution was analyzed by light microscopy. Most of the particles had sizes in the range 10-25 μm . The smallest particles were those of Form IVa, and the largest were those of Form VI. Form I and II gave very similar average results. Form V exhibited the smallest roundness value which explains the best dissolution profile. Form II and IVb were prepared by heating, transforming one polymorph into another, and there was no significant difference in particle size (**Table 5**).

Table 5 The average results of the particle size analysis

Form		Length	Width	Perimeter	Area	Roundness
		[μm]	[μm]	[μm]	[μm] ²	
I	Average	22.7	12.7	68.7	214.9	1.99
	SD $\pm$	13.7	6.6	45.3	300.5	1.13
II	Average	22.5	13.1	77.8	203.1	2.41
	SD $\pm$	12.5	7.0	69.2	201.6	2.18
III	Average	20.4	12.2	62.7	231.8	1.77
	SD $\pm$	13.8	8.7	46.2	336.7	0.75
IVa	Average	10.4	4.6	27.6	33.2	1.85
	SD $\pm$	4.1	1.7	11.2	21.8	0.61
IVb	Average	17.8	6.3	44.3	78.6	2.10
	SD $\pm$	11.4	3.3	28.5	84.4	1.00
V	Average	20.3	14.3	61.9	259.9	1.38
	SD $\pm$	11.8	8.3	40.2	334.7	0.43
VI	Average	109.8	63.5	354.2	5348.4	2.12
	SD $\pm$	62.2	40.2	279.0	5970.1	1.44
VII	Average	31.2	20.0	106.3	476.8	2.23
	SD $\pm$	17.9	11.5	88.9	541.7	1.97

Scanning electron microscopy

The SEM pictures (Fig. 4) reveal that the crystals of the polymorphs present differences in size, morphology and surface. Form I (shock cooling in ethanol) resulted in long needle-shaped crystals. When Form I was heated to 160 °C, the transformation led to a new

morphology. Form II contained prismatic crystals in size range of 25-60 μm . Form III obtained by shock-cooling in isopropanol, 2-butanon, or ethyl acetate gave mostly irregular trapezoid crystals with a smooth surface around 50 μm . Form V crystals obtained by shock cooling in chloroform are around 25 μm , with a nearly ditrigonal morphology. The morphology of Form IVa obtained by shock cooling in methanol and Form IVb obtained by heating of Form IVa were very similar, involving slender ditrigonal and ditetragonal prisms. The crystals of Form IVa were smaller than those of Form IVb. The crystals of Form VI and Form VII were very irregular and varied in size.

FI

FII

FIII

FIVa

FIVb

FV

FVI

FVII

Fig 4 The SEM pictures of the eight forms

Raman spectroscopy

The model drug contains Raman-sensitive C-O-C, C=O, C-N(-N-), methyl and carboxyl groups and cyclohexane, triazole, aromatic and indole rings. There are clear differences between the Raman spectra of the polymorphs in the ranges 3200-2800 cm^{-1} and in particular 1800-900 cm^{-1} (Figs 5 and 6). The Raman spectra of Forms I and IVb demonstrate low Raman intensities (5000 and 900) relative to the other forms (16,000-40,000). Moreover, Form IVb exhibits an unfavourable signal-to-noise ratio and slight fluorescence behaviour. In the range 3000-2800 cm^{-1} , each of the polymorphs gives a characteristic peak, e.g. at 2843 cm^{-1} . Forms III and VII can readily be distinguished through the weak peaks at 3042, 2966 and 3010 and 2990 cm^{-1} , respectively.

In the interval 1800-900 cm^{-1} , further differences can be observed. All the forms have characteristic bands at 1685 (C=O stretching), 1630-1615 (indole ring vibration and hydrazone ring stretching), 1550 (1,2,4,5-tetrasubstituted benzene ring stretching), 1511 (C=C stretching), 1405 (aromatic N=N vibration), 1375 and 1358 (1,2,4-triazole N₂-C₃ and N₄-C₅ stretching), 1208 (aromatic =C-H in-plane deformation), 1050 (cyclohexane C-C skeletal vibration) and 926 cm^{-1} (C-O-C vibration), and additionally at 1473, 1358, 1305 and 1141 cm^{-1} (without assignment). Form V has unique peaks at 1677 (C=O stretching) and 1036 cm^{-1} , while Form VII also has singular peaks at 1694, 1325 and 1247 cm^{-1} .

Muniz-Miranda et al. [162] concluded that the strongest characteristic Raman band of the 1,2,4-triazole ring depends on the distances between the ring atoms and hence on the aromatic ring geometry. No peaks are observed at 1520, 1291, 1259, 1160 and 1068 cm^{-1} in the Raman peaks on the Raman spectra (Figs 6). The ring deformation at 1375 cm^{-1} is attributed to the N₂-C₃ and N₄-C₅ stretching modes of the 1,2,4-triazole ring (Fig. 7). It should be noted that

this type of N₂-C₃/N₄-C₅ bond stretching is a result of a special type of ring deformation, the reason being the substitution on N2, C3 and C5 of the triazole ring.

Fig. 5 Raman spectra of the 8 polymorphs

Fig. 6 Raman spectra of the 8 polymorphs

Fig. 7 Optimized geometry of the 1,2,4-triazole ring (left) and the estimated ring deformation (right) ($\text{N}_2\text{-C}_3$ and $\text{N}_4\text{-C}_5$ stretching)

X-ray powder diffractometry

The XRPD patterns of the eight polymorphs are shown in Fig. 8. The characteristic diffraction peaks are presented in **Table 6**. The data in **Table 6** indicate that the characteristic peaks are situated between 4 and $26^\circ 2\theta$. All the eight polymorphs (with the exception of Forms IVa and IVb) exhibit clear XRPD differences, but these two forms can be well distinguished by other methods, though the crystal structure of Form IVa and IVb were very similar.

Fig. 8 The XRPD patterns of the eight polymorphs

Table 6 The characteristic diffraction peaks of the eight polymorphs

	d (Å)	2 θ (°)	Intensity (CPS)	I/I ₁ (%)		4.816	18.408	3.16	48.0
Form I						4.448	19.947	1.95	29.7
						4.166	21.309	6.59	100.0
	20.054	4.403	10.30	100.0		3.617	24.592	3.05	46.3
	10.388	8.505	0.61	5.9		3.486	25.533	2.51	38.2
	6.640	13.319	0.92	8.9	Form IVb	12.543	7.042	72.40	53.1
	5.926	14.937	0.89	8.7		10.041	8.799	94.50	69.3
	4.995	17.744	1.00	9.7		8.286	10.669	81.20	59.6
	4.156	21.360	6.71	65.0		6.304	14.037	81.90	60.1
	3.398	22.300	2.58	25.0		5.342	16.582	82.10	60.3
	3.463	25.707	1.01	9.8		5.186	17.083	78.50	57.6
Form II						4.444	19.962	72.70	53.4
						4.175	21.263	136.00	100.0
	18.386	4.802	24.50	100.0		4.120	21.550	72.70	53.4
	8.212	10.756	7.99	32.6		3.627	24.521	62.30	45.7
	6.023	14.696	4.10	16.7	Form V	19.665	4.490	0.41	5.1
	5.694	15.549	4.98	20.4		16.458	5.365	8.01	100.0
	5.085	17.428	4.32	17.6	Form VI	10.818	8.167	2.56	32.4
	4.587	19.336	5.51	22.5		8.163	10.830	0.63	8.0
	4.387	20.225	6.25	25.5		5.766	15.355	0.70	8.9
	4.117	21.570	4.85	19.8		5.419	16.343	7.88	100.0
Form III						4.144	21.423	1.67	21.2
						3.819	23.267	4.68	59.4
	10.715	8.245	16.30	100.0		3.743	23.750	0.68	8.6
	9.579	9.224	0.71	4.4		3.627	24.595	3.28	41.6
	9.125	9.685	1.56	9.6		3.121	28.582	0.58	7.4
	8.419	10.498	1.59	9.8		2.406	37.350	0.59	7.4
	8.043	10.992	0.78	4.8	Form VII	17.004	5.193	14.30	100.0
	4.207	21.099	2.09	12.9		10.306	8.573	8.61	60.3
	4.038	21.995	2.49	15.3		9.715	9.096	8.50	59.5
	3.636	24.465	0.98	6.0		8.833	10.006	2.81	19.7
Form IVa						6.208	14.255	4.92	34.4
						5.154	17.191	2.63	18.4
	12.531	7.048	2.28	34.6		4.892	18.121	2.25	15.8
	10.005	8.831	2.09	31.8		4.164	21.321	7.67	53.7
	8.304	10.645	1.93	29.3		3.876	22.925	3.95	27.7
	6.276	14.100	3.03	46.0		3.446	25.835	2.46	17.2

Differential scanning calorimetry

The DSC curves and the relevant data of the eight polymorphs are shown in **Table 7** and Fig. 9. The eight forms give different DSC patterns, except for Form I and II which display a single endothermic peak at about 232 °C (under the DSC conditions, Form I is converted to Form II). Without melting, the transformation of Form I-II could be observed by hot-stage microscopy. Forms III, V, VI, and VII have multiple peaks (endotherms and exotherm) which illustrate transformation by heating via melting. (The presence of solvated morphologies or hydrated ones were excluded by TG, H^1 -NMR and elemental analysis. The drug substance has several solvates, but in this study it was dealt only with “real” polymorphs.) These four polymorphs furnish a common peak at around 232 °C which corresponds to Form II. The melting and recrystallization transformation could also be observed by hot-stage microscopy. Therefore five out of the eight polymorphs transformed to Form II by heating via melting (monotropy) or without it (enantiotropy). For Form IVa, two endothermic peaks are observed at 219 and 227 °C, whereas Form IVb gives only one endothermic peak at 227 °C, which indicates the transformation caused by heating from Form IVa to IVb. The possibility of Form IVa being a solvated or hydrated form of Form IVb was also excluded in the same way as presented above.

Table 7 The relevant DSC data on the 8 polymorphs

Form	T ₁ (°C)	ΔH (J/mol)	T ₂ (°C)	ΔH (J/mol)	T ₃ (°C)	ΔH (J/mol)	T ₄ (°C)	ΔH (J/mol)
I	231.4	-30485.96	–	–	–	–	–	–
II	232.0	-57561.94	–	–	–	–	–	–
III	214.3	-44675.68	219.1	+4089.51	231.8	-15184.30	–	–
IVa	218.7	-36793.19	226.6	-12144.97	–	–	–	–
IVb	227.3	-30615.69	–	–	–	–	–	–
V	130.9	-6603.75	174.8	+28904.52	233.4	-48610.75	–	–
VI	171.4	-15388.15	178.3	+18458.37	231.0	-54238.45	–	–
VII	184.0	-16320.96	188.3	+13053.06	215.4	-605.40	231.4	-22411.97

Fig. 9 The DSC curves of the eight polymorphs

Dissolution rate study

Dissolution rate study was an additional analytical investigation to distinguish the polymorphs generated. Particle size has effect on solubility and dissolution rate, therefore unified particle size is required. Despite of it, in our case there were clear differences in the dissolution between the different polymorphs which was not related to the particle size. In the developed dissolution medium, the rates could be well distinguished (Fig. 10). The fastest dissolution and the largest amount of dissolved compound were seen for Form V, whereas Form III displayed the slowest dissolution, but the lowest amount dissolved belonged to Form IVa. At Forms IVa and IVb a “burst effect” was observed at the beginning of the curve. This is in connection with the small particle size of these two polymorphs. After the 2 h dissolution examination the undissolved remaining of the eight polymorphs were checked by XRPD and no change in the polymorphic forms could be detected in this special dissolution medium.

Fig. 10 The extents of dissolution of the 8 polymorphs

Conclusion

Eight polymorphs for the compound were generated by a polymorph-screening protocol and distinguished by several analytical methods (light microscopy, SEM, Raman-spectroscopy, XRPD and DSC). A special dissolution medium for the screening of our polymorphic modifications was also developed.

5.2. Relative stability examinations

The relative stabilities of the polymorphs generated were investigated by three different analytical methods. All the solids could be stored without any change in morphology for at least 2 years.

Slurry conversion

When the ethanol suspensions were slurried at room temperature Forms I (in 4 days), V (in 17 days), VI (in 10 days) and VII (in a few minutes) turned into Form III, whereas Forms II, III, IVa and IVb did not undergo any change within 130 days. At 50 °C, Form I was transformed to Form III (in 3 days), and Form III was transformed to Form IVb. The other forms

investigated (Forms II, IVa and IVb) remained unchanged during the investigation time (31 days). At 70 °C, Form I turned into Form II, Form III into Form IVb, and Form IVa into Form IVb, while Forms II and IVb did not change during the investigated period (12 hours).

When the polymorphs of the model drug were slurried in silicone oil at 200 °C, only Forms I and III turned into Form II. As expected (due to the generation of Form IVb), Form IVa changed into Form IVb. The other forms were not investigated. No interchange was observed between Forms II and IVb.

Variable-humidity and temperature X-ray powder diffractometry

The *in situ* **VH-XRPD** analyses did not reveal any changes in the eight polymorphs. All of them were stable in the range 20-80 RH% and at 20 RH% in the repeated measurements. Neither the appearance of new reflection peaks nor intensity differences were detected, independently of whether the RH in the measuring chamber was increased or decreased.

By contrast, the *in situ* **VT-XRPD** investigations yielded interesting results as concerns the polymorph transformation screening process. The intensities of the diffraction peaks of Form I decreased continuously during heating. The transformation of Form I started at about 155 °C and was completed at about 190 °C (Fig. 11). After cooling back, the intensities of the diffraction peaks were slightly increased as compared with those of the sample measured at 200 °C. The result of the transformation was Form II. The volume change caused by the temperature change resulted in slight shifts in the reflection peaks. A similar phenomenon was observed in the examination of each polymorph.

Fig. 11 2D VT-XRPD diffractogram of Form I in the interval 30-200 °C

The temperature increase did not cause any phase transition of Form II; this polymorphic form remained stable until melting at 230 °C and after cooling back to 20 °C (Fig. 12). The VT-XRPD investigation indicated that this form was the stable one, and this was confirmed by DSC studies.

Fig. 12 2D VT-XRPD diffractogram of Form II in the interval 30-200 °C

The phase transition of Form III started at about 215 °C. After recooling, the new low-intensity reflection peaks measured at 230 °C were increased significantly; Form II was identified. The shift in the lower-intensity reflection peaks at 2θ values of about 16-17° and the higher-intensity peaks at 2θ values of around 25° started at 160 °C, which indicated the earlier onset of this phase transition with some content of Form I (Fig. 13).

Fig. 13 2D VT-XRPD diffractogram of Form III in the interval 30-230 °C

Increase of the temperature did not cause any changes in the Form IVa and IVb polymorphs up to 225 °C. Further increase of the temperature resulted in the melting of these samples, and after cooling back to 30 °C the samples became amorphous. Repeated measurements on the following day verified the persistence of the amorphous form. Because of the limitations of the VT-XRPD instrument, a sufficiently high temperature for the transformation of Form IVa to Form IVb could not be attained. During the heating of Form V, the diffraction peaks disappeared at about 145 °C. Thermomicroscopic studies confirmed that this phenomenon was caused by the melting process. Crystallization of the melt started at about 150 °C. The diffractogram of this new form (Intermediate I) differed from that of any other polymorph. During further heating of the material, a phase-transition process started at about 190 °C, resulting in a second new form (Intermediate II), and Form II finally crystallized at about 220 °C. The diffraction peaks of the material cooled down to 20 °C were those of pure Form II (Figs 14 and 15).

Fig. 14 2D VT-XRPD diffractogram of Form V in the interval 30-230 °C

Fig. 15 VT-XRPD diffractograms of Form V at several temperatures

During the heating of Form VI, two phase-transition processes were detected, with onset temperatures of about 175 °C and 190 °C. The result of the first process was the previously detected Intermediate I, while the second series of peaks were those of Form II (Fig. 16).

Fig. 16 2D VT-XRPD diffractogram of Form VI in the interval 30-230 °C

Finally, similarly to Form I, Form VII was transformed to Form II. The onset temperature of the phase transition was about 190 °C.

Intermediates I and II could not be prepared in this investigation: although the heating was stopped at the given temperature, the transformation process continued. These two forms are unstable forms; all attempts to isolate them resulted in the stable Form II.

Differential scanning calorimetry

DSC investigations of all the polymorphic forms were performed at several heating rates. The results confirmed the findings of the XRPD investigations. The DSC studies carried out at different heating rates led to different results. Both the low (1 °C min⁻¹) and the high (30 °C min⁻¹) heating rate studies provided additional information on the phase-transition processes.

The polymorph phase-transition peak of Form I could not be detected at low heating rate. However, a heating rate of 30 °C min⁻¹ gave rise to a small exothermic peak at an onset temperature of 190 °C, which indicated that Form I was transformed into the more stable Form II. It was also observed that a higher heating rate generally caused the peaks to shift towards higher temperatures (Fig. 17). A similar phenomenon was observed for Form VII.

Fig. 17 DSC curves of Form I at different heating rates

Depending on the heating rate, the DSC curves of Form II contained one endothermic peak at about 231-235 °C, which corresponded to the melting point of the most stable Form II.

The phase-transition process of Form III to Form II was seen in a DSC curve at a lower heating rate (an endothermic peak at 213 °C and an exothermic peak at 214-215 °C). These processes could not be distinguished at the highest heating rate. The slow phase-transformation process observed between 160 and 215 °C in the XRPD investigations was not detected in the DSC curves (Fig. 18)

Fig. 18 DSC curves of Form III at different heating rates

In the DSC curves of Form V, the endothermic peak of melting was identified at about 132-146 °C. At heating rates of 1 and 10 °C min⁻¹, only the first phase-transition process was observed, which shifted towards higher temperatures on increase of the heating rate. The two phase-transition processes were seen only in the DSC curve at higher heating rate (Fig. 19).

The phase-transition process of Form VI was detected in the DSC curve at a heating rate of 10 °C min⁻¹. The endothermic and exothermic pair at about 178 and 186 °C corresponded to the appearance of Intermediate I. The phase transition was not detected at a heating rate of 1 °C min⁻¹, while the subprocesses could not be separated at 30 °C min⁻¹.

Fig. 19 DSC curves of Form V at different heating rates

The MTDSC investigations did not provide more information on the relative stability of the polymorphs, and these results are therefore not presented in my PhD work.

The enthalpies of Forms III, IVa, VI and VII were merely estimated, in view of the inability to obtain a single melting endotherm. The enthalpy of the melting endotherm for Form II proved to be the highest, and that of Form V the lowest (Table 7). Form II was not transformed to Form IVb or *vice versa*.

Conclusion

During the relative stability studies, the isothermal suspension equilibration and VT-XRPD methods led to different phase-transformation processes for some polymorphic forms. These phase-transition processes were confirmed by DSC studies carried out at different heating rates. The VT-XRPD investigations demonstrated the formation of two new unstable intermediate forms (from Forms V and VI). Forms IVa and IVb resulted in amorphous materials. Variation of the relative humidity did not influence the polymorphic transformations. It was concluded that Forms I, III, IVa, V, VI and VII are metastables, while Form IVb has almost the same stability as that of Form II because the transformation of Form IVb to Form II (and *vice versa*) has not been achieved to date. The enthalpy data, the

isothermal suspension equilibration investigations and the XRPD results confirmed that the thermodynamically stable polymorph is Form II.

5.3. The influence of sucrose esters on the polymorphism of the model drug

In the first five samples evaluated, the ratio of the model drug and the SE was 1:1. In each case, XRPD was used to identify the forms and to compare the samples containing SE with the pure polymorphs. Two SEs, sucrose laurate L 595 (HLB 5) and sucrose oleate O 1570 (HLB 15), were found to modify the original Form IVa to Form III (Fig. 20). The other three selected SEs (S 370, S 1570 and D 1216) did not affect the polymorphism.

In the second step, the influence of L 595 and O 1570 on the polymorphism was examined at four other concentration ratios. Both SEs gave the same results: the transformation of Form IVa to Form III occurred except when the quantity of the SE was 4 times that of the model drug in the sample. In that case, no characteristic peaks were seen in the X-ray powder diffractograms, which can be explained by the crystallization-inhibiting property of these SEs.

Fig. 20 The XRPD patterns of the samples crystallized in the presence of SE O-1570 or L-595 compared with the original Forms III and IVa

6. SUMMARY

A polymorph screening investigation on a failed drug candidate was carried out to develop an appropriate method with which to distinguish the polymorphs generated after their dissolution, to investigate the relative stabilities of the polymorphs and to study the effects of SEs as additives during the screening process.

A. Preparation and identification of polymorphs

A failed drug candidate obtained from Sanofi Pharmaceutical Company (Hungary) was investigated to explore its polymorphism by means of analytical examinations. The primary objective was to develop a polymorph screening method. Through crystallization and transformation methods, eight polymorphs were obtained and were characterized by light microscopy, SEM, XRPD, DSC and Raman spectroscopy. The different polymorphs exhibit different solubilities, which affects the bioavailability of the drug, and it is therefore important to perform dissolution studies with the different polymorphs. Because of the poor water-solubility of the model drug, a special dissolution medium was developed in which the eight pure polymorphs could be well distinguished.

XRPD is always the definitive method for the identification of polymorphs, but in some cases (Forms IVa and IVb) other analytical examinations were also required to characterize the individual forms.

B. Analysis of the polymorph changes

After the preparation and structural characterization of the polymorphs, their relative stabilities were also studied by an isothermal suspension equilibration method, VH-XRPD, VT-XRPD and DSC.

For the isothermal suspension equilibration study the Avantium Crystal 16 automatic laboratory reactor system was complemented with XRPD to identify the morphologies. Its main advantage that the effects of solvent- and temperature-mediated polymorphic transformations can be investigated.

In DSC tests, the application of different heating rates is recommended in order to obtain appropriate information relating to the details of phase-transformation processes. The literature findings indicate that the use of MTDSC may also give additional data, but in our case it did not provide more information.

The model drug was not sensitive to humidity, and variation of the relative humidity therefore had no influence on the polymorphic transformations.

C. The influence of SEs on polymorphism

D. Practical relevance of the results

is essential to investigate polymorphism for both economic and therapeutic reasons. The accurate establishment of thermodynamic stability relations is also very important, because unexpected polymorphs can appear. VT-XRPD can be a very useful technique for such stability studies.

In the course of the polymorph screening process, it is sufficient to use only conventional crystallization methods, where the solvent and the temperature affect the formation of the crystals. The effects of the different additives used during the pharmaceutical production, and especially the surfactants, such as the SEs, must also be considered. The use of SEs in the pharmaceutical industry is increasing, because of their advantageous properties, such as solubility enhancement or emulsification. As this PhD work reveals, these commonly used additives can influence polymorphic phase-transformations, which can lead to undesirable changes in the product.

REFERENCES

1. Hilfiker, R.: Polymorphism: in the Pharmaceutical Industry, Wiley-VCH, Weinheim, 2006.
2. Theyer, A. M.: Chemical & Engineering News - Cover story; 85, 25 (2007) 17-30.
3. Byrn, S. R.; Pfeiffer, R.; Ganey, M.; Hoiberg, C.; Poochikian, G.: Pharmaceutical solids: a strategic approach to regulatory considerations, *Pharm. Res.*, 12 (1995) 945-954.
4. Huang, L.-F.; Tong, W.-Q.: Impact of solid state properties on developability assessment of drug candidates, *Adv. Drug Delivery Rev.*, 56 (2004) 321-334.
5. Raw, A. S.; Furness, M. S.; Gill, D. S.; Adams, R. C.; Holcombe, F. O. Jr.; Yu, L. X.: Regulatory considerations of pharmaceutical solid polymorphism in Abbreviated New Drug Applications (ANDAs). *Adv. Drug Delivery Rev.*, 56 (2004) 397-414.
6. Morissette, S. L. et al.: High-throughput crystallization: polymorphs, salts, co-crystals and solvates of pharmaceutical solids, *Adv. Drug Delivery Rev.*, 56 (2004) 275-300.
7. Byrn, S. R.; Pfeiffer, R. R.; Stowell, J. G.: Solid-State Chemistry of Drugs, SSCI, West Lafayette, IN, 1999.
8. Brittain, H. G.: Polymorphism in Pharmaceutical Solids; Marcel Dekker, New York, 1999.
9. Davey, R.; Garside, J.: From Molecules to Crystallizers, Oxford University Press, New York, 2000.
10. Giron, D.: Investigations of polymorphism and pseudo-polymorphism in pharmaceuticals by combined thermoanalytical techniques, *J. Therm. Anal. Cal.*, 64 (2001) 37-60.
11. Jackson, K.; Young, D.; Pant, S.: Drug-excipient interactions and their affect on absorption, *Pharmaceutical Science & Technology Today*, Vol. 3., Is. 10. (2000) 336-345.
12. Gu, C. H.; Chatterjee, K.; Jr. Young, V.; Grant, D. J. W.: Stabilization of a metastable polymorph of sulfamerazine by structurally related additives, *Journal of Crystal Growth* 235 (2002) 471-481.
13. Hilfiker, R.; Blatter, F.; Raumer, M.: Polymorphism: in the Pharmaceutical Industry, Relevance of Solid-state Properties for Pharmaceutical Products, Wiley-VCH, Weinheim, 2006. pp. 1-19.
14. Vrcelj, R. M.; Gallagher, H. G.; Sherwood, J. N.: Polymorphism in 2, 4, 6-trinitrotoluene crystallized from solution, *J. Am. Chem. Soc.*, 123 (2001) 2291.
15. Mitsubishi- Kagaku Foods Corporation, 1982. Ryoto Sugar Ester Technical Information. Nonionic surfactant/Sucrose fatty acid ester/Food additive
16. Akoh, C. C., Emulsification properties of polyesters and sucrose ester blends I: Carbohydrate fatty acid polyesters, *J. Am. Oil Chem. Soc.*, 69 (1992) 9-13.
17. Mutoh, T.; Kubouchi, H.; Noda, M.; Shiinoki, Y.; Matsumura, Y.: Effect of oil-soluble emulsifiers on solidification of thermally treated creams, *Int. Dairy J.*, 17 (2007) 24-28.

18. Oh, J.; Swanson, B. G.: Polymorphic transitions of cocoa butter affected by high hydrostatic pressure and sucrose polyesters, *J. Am. Oil. Chem. Soc.*, 83 (2006) 1007-1014.
19. Garti, N.; Aserin, A.; Fanun, M.: Non-ionic sucrose esters microemulsions for food applications. Part 1. Water solubilization, *Coll. Surf. A.*, 164 (2000) 27-38.
20. Kato, A.; Arima, K.: Inhibitory effect of sucrose ester of lauric acid on the growth of *Escherichia coli*, *Biochem. Biophys. Res. Commun.*, 42 (1971) 596-601.
21. Awad, T.; Kato, S.: Acceleration of crystallization of palm cernel oil in oil-in-water emulsion by hydrophobic emulsifier additives, *Coll. Surf. B.*, 25 (2002) 45-53.
22. Szűts, A.; Láng, P.; Ambrus, R.; Kiss, L.; Deli, A. M.; Szabó-Révész, P.: Applicability of sucrose laurate as surfactant in solid dispersions prepared by melt technology, *Int. J. Pharm.*, 410 (2011) 107-110.
23. Farkas, B.; Révész, P.: *Kristályosítástól a tablettázásig*, Universitas: Szeged, 2007.
24. Specification: Test procedures and acceptance criteria for new drug substances and new drug products: chemical substances Q6A, part of U. S. Food and Drug Administration.
http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q6A/Step4/Q6Astep4.pdf (accessed 26.11.2015).
25. Purohit, R.; Venugopalan, P.: *Polymorphism: An Overview*, Resonance, 2009.
26. Dunitz, J. D.; Bernsterin J.: Disappearing polymorphs. *Acc Chem Res*, 28 (1995) 193-200.
27. Lancaster, R. W.; Karamertzanis, P. G.; Hulme, A. T. et al.: The polymorphism of progesterone: stabilization of a 'disappearing' polymorph by co-crystallization, *J Pharm Sci*, 96 (2007) 3419-3431.
28. Lancaster, R. W.; Harris, L. D.; Pearson D.: Fifty-year old samples of progesterone demonstrate the complex role of synthetic impurities in stabilizing a metastable polymorph, *Cryst Eng Comm*, 13 (2011) 1775-1777.
29. Lee, E. H.: A practical guide to pharmaceutical polymorph screening & selection, *Asian Journal of Pharmaceutical Sciences*, 9 (2014) 163-175.
30. Li, Y.; Chow, P. S.; Tan, R.B.H.: Quantification of polymorphic impurity in anenantiotropic polymorph system using differential scanning calorimetry, X-ray powder diffraction and Raman spectroscopy, *Int. J. Pharm.* 415 (2011) 110-118.
31. Van Eupen, J. Th. H.; Westheim, R.; Deij, M. A.; Meekes, H.; Bennema, P.; Vlieg, E.: The solubility behaviour and thermodynamic relations of the three forms of Venlafaxine free base, *Int. J. Pharm.* 368 (2009) 146-153.
32. Bernstein, J.: *Polymorphism in Molecular Crystals*, Oxford Science Publications, Oxford, 2002.
33. Thun, J.; Seyfarth, L.; Senker, J.; Dinnebier, R. E.; Breu, J.: Polymorphism in Benzamide: solving a 175-year-old riddle, *Angew. Chem., Int. Ed.* 46 (2007) 6729-6731.
34. Chemburkar, S. R. et al.: Dealing with the impact of Ritonavir polymorphs on the late stages of bulk drug process development, *Org. Process Res. Dev.*, 4 (2000) 413-417.

35. McCrone, W. C.: Polymorphism. In: Fox D, Labes MM, Weissberger M, editors. *Physics and chemistry of the organic solid state*, vol. 2. p.725., Wiley Interscience, New York, 1965.
36. Aaltonen, J.; Allesø, M.; Mirza, S.; Koradia, V.; Gordon, K. C.; Rantanen, J.: Solid form screening-A review, *Eur. J. Pharm. Biopharm.* 71 (2009) 23-37.
37. Singhal, D.; Curatolo, W.: Drug polymorphism and dosage form design: a practical perspective, *Adv. Drug Del. Rev.* 56 (2004) 335-347.
38. Hilfiker, R.; De Paul, S. M.; Szelagiewcz, M.: Approaches to polymorphism screening, in: R. Hilfiker (Ed.), *Polymorphism in the Pharmaceutical Industry*, Wiley-VCH Verlag GmbH, Weinheim, 2006, pp. 287-308.
39. Park, A. et al.: New solid-state chemistry technologies to bring better drugs to market: knowledge-based decision making, *Expert Opin. Drug Discov.* 2 (2007) 145-154.
40. Florence, A. J.; Johnston A.; Price, S. L. et al.: An automated parallel crystallization search for predicted crystal structures and packing motifs of carbamazepine, *J. Pharm. Sci.*, 95 (2006) 1918-1930.
41. Almarson, Ö.; Hickey, M. B.; Peterson, M. L. et al.: High-throughput surveys of crystal form diversity of highly polymorphic pharmaceutical compounds, *Cryst. Growth Des.*, 3 (2003) 927-933.
42. Morrisette, S. L.; Soukasene, S.; Levinson, D. et al.: Elucidation of crystal form diversity of the HIV protease inhibitor ritonavir by high-throughput crystallization, *Proc. Natl. Acad. Sci.*, 100 (2003) 2180-2184.
43. Morrisette, S. L.; Almarsson, Ö.; Peterson, M. L. et al.: High-throughput crystallization: polymorphs, salts, co-crystals and solvates of pharmaceutical solids, *Adv. Drug Deliv. Rev.*, 56 (2004) 275-300.
44. Leterson, M. L.; Morrisette, S. L.; McNulty, C. et al.: Iterative high-throughput polymorphism studies on acetaminophen and an experimentally derived structure for Form III, *J. Am. Chem. Soc.*, 124 (2002) 10958-10959.
45. Krc, Jr. J.: Crystallographic properties of flufenamic acid, *Microscope*, 25 (1976) 31-45.
46. Wu, T; Yu, L.: Origin of enhanced crystal growth kinetics near T_g probed with indomethacin polymorphs, *J. Phys. Chem. B.*, 110 (2006) 15694-15699.
47. Sun, Y.; Xi, H.; Chen, S. et al.: Crystallization near glass transition: transition from diffusion-controlled to diffusionless crystal growth studied with seven polymorphs, *J. Phys. Chem. B.*, 112 (2008) 5594-5601.
48. Zhu, L.; Wong, L.; Yu, L.: Surface-enhanced crystallization of amorphous nifedipine, *Mol. Pharm.*, 5 (2008) 921-926.
49. Albers, D.; Galgoci, M.; King, D. et al.: Characterization of the polymorphic behavior of an organic compound using a dynamic thermal and X-ray powder diffraction technique, *Org. Process Res. Dev.*, 11 (2007) 846-860.
50. Campeta, A. M.; Chekal, B. P.; Abramov, Y. A. et al.: Development of a targeted polymorph screening approach for a complex polymorphic and highly solvating API, *J. Pharm. Sci.*, 99 (2010) 3874-3886.

51. Rengarajan, G. T.; Enke, D.; Steinhart, M. et al.: Size-dependent growth of polymorphs in nanopores and Ostwald's step rule of stages, *Phys. Chem. Chem. Phys.*, 13 (2011) 21367-21374.
52. Beiner, M.; Rengarajan, G. T.; Pankaj, S. et al.: Manipulating the crystalline state of pharmaceuticals by nanoconfinement, *Nano Lett.*, 7 (2007) 1381-1385.
53. Ha, J. M.; Wolf, J. H.; Hillmyer, M. A. et al.: Polymorph selectivity under nanoscopic confinement. *J. Am. Chem. Soc.*, 126 (2004) 3382-3383.
54. Beckmann, W.; Nickisch, K.; Budde, U.: Development of a seeding technique for the crystallization of the metastable A modification of abecarnil, *Org. Process Res. Dev.*, 2 (1998) 298-304.
55. Friscic, T.; MacGillivray, L. R.: Engineering cocrystal and polymorph architecture via pseudoseeding, *Chem. Commun.*, (2009) 773-775.
56. Gu, C. H.; Young, Jr., V.; Grant, D. J. W.: Polymorph screening: influence of solvents on the rate of solvent-mediated polymorphic transformation, *J. Pharm. Sci.*, 90 (2001) 1878-1890.
57. Qu, H.; Louhi-Kultanen, M.; Kallas, J.: Solubility and stability of anhydrate/hydrate in solvent mixtures, *Int. J. Pharm.*, 321 (2006) 101-107.
58. Zhu, H.; Grant, D. J. W.: Influence of water activity in organic solvent + water mixtures on the nature of the crystallizing drug phase. 2. Ampicillin, *Int. J. Pharm.*, 139 (1996) 33-43.
59. Zhu, H.; Yuen, C.; Grant, D. J. W.: Influence of water activity in organic solvent + water mixtures on the nature of the crystallizing drug phase. 1. Theophylline, *Int. J. Pharm.*, 135 (1996) 151-160.
60. Lee, E. H.; Byrn, S. R., Stabilization of metastable flufenamic acid by inclusion of mefenamic acid: solid solution or epilayer?: *J. Pharm. Sci.*, 99 (2010) 4013-4022.
61. Braga, D.; Grepioni, F.; Maini, L.: The growing world of crystal forms, *Chem. Commun.*, 46 (2010) 6232-6242.
62. Lin, S. Y.: An overview of famotidine polymorphs: solid-state characteristics, thermodynamics, polymorphic transformation and quality control, *Pharm Res.*, 31 (7) (2014) 1619-1631.
63. Chyall, L. J.; Tower, J. M.; Coates, D. A. et al.: Polymorph generation in capillary spaces: the preparation and structural analysis of a metastable polymorph of nabumetone, *Cryst. Growth Deg.*, 2 (2002) 505-510.
64. Hilden, J. L.; Reyes, C. E.; Kelm, M. J. et al.: Capillary precipitation of a highly polymorphic organic compound, *Cryst. Growth Des.*, 3 (2003) 921-926.
65. Otsuka, M.; Ofusa, T.; Matsuda, Y.: Effect of environmental humidity on the transformation pathway of carbamazepine polymorphic modifications during grinding, *Colloids Surf. B.*, 13 (1999) 263-273.
66. Matuda, Y.; Tatsumi, E.; Chiba, E. et al.: Kinetic study of the polymorphic transformations of phenylbutazone, *J. Pharm. Sci.*, 73 (1984) 1453-1460.
67. Terada, K.; Kurobe, H.; Ito, M. et al.: Polymorphic and pseudomorphic transformation behavior of acyclovir based on thermodynamics and crystallography, *J. Therm. Anal. Calorim.*, 113 (2013) 1261-1267.

68. Lee, J.; Boerrigter, S. X. M.; Jung, Y. W. et al.: Organic vapor sorption method of isostructural solvates and polymorph of tenofovir disoproxil fumarate, *Eur. J. Pharm. Sci.*, 50 (2013) 253-262.
69. Carter, P. W.; Ward, M. D.: Topographically directed nucleation of organic crystals on molecular single-crystal substrates, *J. Am. Chem. Soc.*, 115 (1993) 11521-11535.
70. Bonafede, S. J.; Ward, M. D.: Selective nucleation and growth of an organic polymorph by ledge-directed epitaxy on a molecular crystal substrate, *J. Am. Chem. Soc.*, 117 (1995) 7853-7861.
71. Mitchell, C.A.; Yu, L.; Ward, M. D.: Selective nucleation and discovery of organic polymorphs through epitaxy with single crystal substrates, *J. Am. Chem. Soc.*, 123 (2001) 10830-10839.
72. Frincu, M. C.; Sharpe, R. E.; Swift, J. A.: Epitaxial relationships between cholesterol crystals and mineral phases: implication for human disease, *Cryst. Growth Des.*, 4 (2004) 223-226.
73. Seaton, C. C.; Parkin, A.; Wilson, C. C. et al.: Growth of an organic co-crystal upon a component subphase, *Cryst. Growth. Des.*, 8 (2008) 363-368.
74. Lee, E. H.; Boerrigter, S. X. M.; Byrn, S. R.: Epitaxy of a structurally related compound on the (100) faces of flufenamic acid form I and III single crystals, *Cryst. Growth Des.*, 10 (2010) 518-527.
75. Addadi, L.; Berkovitch-Yellin, Z.; Weissbuch, I. et al.: Growth and dissolution of organic crystals with "tailor-made" inhibitors-implications in stereochemistry and materials science, *Angew Chem. Int. Ed. Engl.*, 24 (1981) 466-485.
76. Addadi, L.; Weiner, S.: Control and design principles in biological mineralization, *Angew. Chem. Int. Ed. Engl.*, 31 (1992) 153-169.
77. Leiserowitz, L.; Nader, F.: The molecular packing modes and the hydrogen-bonding properties of amide: dicarboxylic acid complexes, *Acta Cryst.*, B33 (1977) 2719-2733.
78. Addadi, L.; Lahav, M.: Toward the planning and execution of an "absolute" asymmetric synthesis of chiral dimmers and polymers with quantitative enantiomeric yield, *Pure Appl. Chem.*, 51 (1979) 1269-1284.
79. Mil, van J.; Gati, E.; Addai, L. et al.: Useful impurities for optical resolutions. 1. On the crystallization of photopolymerizing dienes in the presence of their chiral topochemical products, *J. Am. Chem. Soc.*, 103 (1981) 1248-1249.
80. Addadi, L.; Mil, van J.; Lahav, M.: Useful impurities for optical resolutions. 2. Generality and mechanism of the rule of reversal, *J. Am. Chem. Soc.*, 103 (1981) 1249-1251.
81. Addadi, L.; Gati, E.; Lahav, M.: Useful impurities for optical resolutions 3. An improved Pasteur-type resolution of conglomerates and a new empirical method for assignment of absolute configuration, *J. Am. Chem. Soc.*, 103 (1981) 1251-1252.
82. Addadi, L.; Mil, van J.; Lahav, M.: Engineering of chiral crystals for asymmetric ($2\pi+2\pi$) photopolymerization. Execution of an "absolute" asymmetric synthesis with quantitative enantiomeric yield, *J. Am. Chem. Soc.*, 104 (1982) 3422-3429.
83. Mil van J.; Addadi, L.; Gati, E.; et al.: Attempted amplification of optical activity by crystallization of chiral crystals of photopolymerizing dienes in the presence of their topochemical products, *J. Am. Chem. Soc.*, 104 (1982) 3429-3434.

84. Addadi, L.; Weinsein, S.; Gati, E. et al.: Resolution of conglomerates with the assistance of tailor-made impurities. Generality and mechanistic aspects of the “rule of reversal”. A new method for assignment of absolute configuration, *J. Am. Chem. Soc.*, 104 (1982) 4610-4617.
85. Weissbuch, I.; Popovitz-Biro, R.; Lahav, M. et al.: Understanding and control of nucleation, growth, habit, dissolution and structure of two- and three-dimensional crystals using ‘tailor-made’ auxiliaries, *Acta Cryst.*, B51 (1995) 115-148.
86. Weissbuch, I.; Lahav, M.; Leiserowitz, L.: Toward stereochemical control, monitoring, and understanding of crystal nucleation, *Cryst. Growth Des.*, 3 (2003) 125-150.
87. Davey, R. J.; Blagden, N.; Potts, G. D. et al.: Polymorphism in molecular crystals: stabilization of a metastable form by conformational mimicry, *J. Am. Chem. Soc.*, 119 (1997) 1767-1772.
88. Lee, E. H.; Byrn, S. R.; Carvajal, M. T.: Additive-induced metastable single crystal of mefenamic acid, *Pharm. Res.* 23 (2006) 2375-2380.
89. Mattei, A.; Li, T.: Polymorph formation and nucleation mechanism of tolfenamic acid in solution: an investigation of pre-nucleation solute association, *Pharm. Res.*, 29 (2012) 460-470.
90. Behme, R. J.; Brooke, D.: Heat of fusion measurement of a low melting polymorph of carbamazepine that undergoes multiple-phase changes during differential scanning calorimetry, *J. Pharm. Sci.*, 80 (1991) 986-990.
91. Park, Y.; Lee, J.; Lee, S. H. et al.: Crystal structures of tetramorphic forms of donepezil and energy/temperature phase diagram via direct heat capacity measurements, *Cryst. Growth Des.*, 13 (2013) 5450-5458.
92. Pasquali, I.; Bettini, R.; Giordano, F.: Supercritical fluid technologies: an innovative approach for manipulating solid-state of pharmaceuticals, *Adv. Drug. Deliv. Rev.*, 60 (2008) 399-410.
93. Tozuka, Y.; Kawada, D.; Oguchi, T. et al.: Supercritical carbon dioxide treatment as a method for polymorph preparation of deoxycholic acid, *Int. J. Pharm.*, 263 (2003) 45-50.
94. Bettini, R.; Bonassi, L.; Castoro, V. et al.: Solubility and conversion of carbamazepine polymorphs in supercritical carbon dioxide, *Eur. J. Pharm. Sci.*, 13 (2001) 281-286.
95. Mooij, W. T. M.; Eijck, van B. P.; Kroon, J.: Ab initio crystal structure predictions for flexible hydrogen-bonded molecules, *J. Am. Chem. Soc.*, 122 (2000) 3500-3505.
96. Price, S. L.: The computational prediction of pharmaceutical crystal structures and polymorphism, *Adv. Drug Deliv. Rev.*, 56 (2004) 301-319.
97. Ouvrard, C.; Price, S. L.: Toward crystal structure prediction for conformationally flexible molecules: the headaches illustrated by aspirin, *Cryst. Growth Des.*, 4 (2004) 1119-1127.
98. Nowell, H.; Frampton, C. S.; Waite, J. et al.: Blind crystal structure of a novel second polymorph of 1-hydroxy-7-azabenzotriazole, *Acta Cryst.*, B62 (2006) 642-650.
99. Braun, D. E.; Karamertzanis, P. G.; Price, S. L.: Which, if any, hydrates will crystallize? Predicting hydrate formation of two dihydroxybenzoic acids, *Chem. Commun.*, 47 (2011) 5443-5445.

100. Sun, C.: Solid-state properties and crystallization behavior of PHA-739521 polymorphs, *Int. J. Pharm.*, 319 (2006) 114–120.
101. Gu, C.; Li, H.; Gandhi, R. B. et al.: Grouping solvents by statistical analysis of solvent property parameters: implication to polymorph screening, *Int. J. Pharm.*, 283 (2004) 117-125.
102. Volmer, M.: *Die chemische reaktion. Bd. IV. Kinetik der phasenbildung*, Steinkopf, Leipzig, 1939.
103. Behme, R. J.; Brooke, D.: Heat of fusion measurement of a low melting polymorph of carbamazepine that undergoes multiple-phase changes during differential scanning calorimetry, *J. Pharm. Sci.*, 80 (1991) 986-990.
104. Cardew, P. T.; Davey, R. J.: The kinetics of solvent-mediated phase transformation, *Proc. R. Soc. Lond. A*, 398 (1985) 415-428.
105. Davey, R. J.; Cardew, P. T.; McEwan, D. et al.: Rate controlling processes in solvent-mediated phase transformations, *J. Cryst. Growth*, 79 (1986) 648-653.
106. Park, Y.; Lee, J.; Lee, S. H. et al.: Crystal structures of tetramorphic forms of donepezil and energy/temperature phase diagram via direct heat capacity measurements, *Cryst. Growth Des.*, 13 (2013) 5450-5458.
107. Florence, A. J.; Johnston, A.; Price, S. L. et al.: An automated parallel crystallization search for predicted crystal structures and packing motifs of carbamazepine, *J. Pharm. Sci.*, 95 (2006) 1918-1930.
108. Yamauchi, M.; Lee, E. H.; Otte, A. et al.: Contrasting the surface and bulk properties of anhydrate and dehydrated hydrate materials, *Cryst. Growth Des.*, 11 (2011) 692-698.
109. Mirza, S.; Miroshynik, I.; Rantanen, J. et al.: Solid-state properties and relationship between anhydrate and monohydrate of baclofen, *J. Pharm. Sci.* 96 (2007) 2399-2408.
110. Chen, L. R.; Young, Jr. V. G.; Lechuga-Ballesteros, D. et al.: Solidstate behavior of cromolyn sodium hydrates, *J. Pharm. Sci.*, 88 (1999) 1191-1200.
111. Lee, E. H.; Smith, D. T.; Fanwick, P. E. et al.: Characterization and anisotropic lattice expansion/contraction of polymorphs of tenofovir disoproxil fumarate, *Cryst. Growth Des.*, 10 (2010) 2314-2322.
112. Lee, J.; Boerrigter, S. X. M.; Jung, Y. W. et al.: Organic vapor sorption method of isostructural solvates and polymorph of tenofovir disoproxil fumarate, *Eur. J. Pharm. Sci.*, 50 (2013) 253-262.
113. Campeta, A. M.; Chekal, B. P.; Abramov, Y. A. et al., Development of a targeted polymorph screening approach for a complex polymorphic and highly solvating API, *J. Pharm. Sci.*, 99 (2010) 3874-3886.
114. Thallapally, P. K. et al.: Polymorphism of 1,3,5-Trinitrobenzene Induced by a Trisindane Additive, *Angew. Chem.*, 116 (2004) 1169-1169.
115. Baynes, B. M.; Trout, Bernhardt, L.: Rational Design of Solution Additives for the Prevention of Protein Aggregation, *Biophys. J.* 87 (2004) 1631-1639.
116. Lee, E. H.: Effects of additives on crystallization, polymorphic transformation, and solubility, PhD thesis, Purdue, 2007.
117. Hutchinson, A.: The Effect of Additives on the Growth of Benzophenone, PhD thesis, Manchester, 2014.

118. Weissbuch, I.; Popovitz-Biro, R.; Lahav, M. et al.: Understanding and control of nucleation, growth, habit, dissolution and structure of two- and three-dimensional crystals using 'tailor-made' auxiliaries. *Acta Cryst*, 51 (1995) 115-148.
119. Awad, T.; Sato, K.: Acceleration of crystallisation of palm kernel oil in oil-in-water emulsion by hydrophobic emulsifier additives, *Colloids and Surfaces B: Biointerfaces* 25 (2002) 45-53.
120. Szűts, A.; Láng, P.; Ambrus, R.; Kiss, L.; Deli, A. M.; Szabó-Révész, P.: Applicability of sucrose laurate as surfactant in solid dispersions prepared by melt technology, *Int. J. Pharm.*, 410 (2011) 107-110.
121. Abd-Elbary, A.; El-laithy, H. M.; Tadros, M. I.: Sucrose stearate-based proniosome derived niosomes for the nebulisable delivery of cromolyn sodium, *Int. J. Pharm.*, 357 (2008) 189-198.
122. Csóka, G.; Marton, S.; Zelko, R.; Otomo, N.; Antal, I.: Application of sucrose fatty acid esters in transdermal therapeutic systems, *Eur. J. Pharm. Biopharm.*, 65 (2007) 233-237.
123. Ganem Quintanar, A.; Quintanar-Guerrero, D.; Falson-Rieg, F.; Buri, P.: Ex vivo oral mucosal permeation of lidocaine hydrochloride with sucrose fatty acid esters as absorption enhancers, *Int. J. Pharm.*, 173 (1998) 203-210.
124. Okamoto, H.; Takashi, S.; Kazumi, D.: Effect of sucrose fatty acid esters on transdermal permeation of lidocaine and ketoprofen, *Biol. Pharm. Bull.*, 28 (2005) 1689-1694.
125. Otomo, N.: Basic properties of sucrose fatty acid esters and their applications. In: Hayes, D.G., Kitamoto, D., Solaiman, D.K.Y., Ashby, R.D. (Eds.), *Biobased Surfactants and Detergents: Synthesis, Properties, and Applications*. AOCS Press, Urbana, Illinois, (2009) pp. 275-298.
126. Ntawukulilyayo, J. D.; Bouckaert, S.; Remon, J. P.: Enhancement of dissolution rate of nifedipine using sucrose ester coprecipitates, *Int. J. Pharm.*, 93 (1993) 209-214.
127. Shibata, D.; Shimada, Y.; Yonezawa, Y.; Sunada, H.; Otomo, N.; Kasahara, K.: Application and evaluation of sucrose fatty acid esters as lubricants in the production of pharmaceuticals, *J. Pharm. Sci. Technol.*, 62 (2002) 133-145.
128. Akoh, C. C.: Emulsification Properties of Polyesters and Sucrose Ester Blends I: Carbohydrate Fatty Acid Polyesters, *JAOCS*, Vol. 69, No. 1 (1992).
129. Otsuka, M.; Ofusa, T.; Matsuda, Y.: Dissolution improvement of water-insoluble glybuzole by co-grinding and co-melting with surfactants and their physicochemical properties, *Colloids Surf. B.*, 10 (1998) 217-226.
130. Aoshima, H.; Miyagisnima, A.; Nozawa, Y.; Sadzuka, Y.; Sonobe, T.: Glycerin fatty acid esters as a new lubricant of tablets, *Int. J. Pharm.*, 293 (2005) 25-34.
131. Carlton, R. A.: *Pharmaceutical Microscopy*, Springer-Verlag, New York, 2011.
132. Chadha, R.; Arora, P.; Bhandari, S.; Bala, M.: Thermomicroscopy and its pharmaceuticals applications, in: Mendez-Vilas, A. (ed.), *Current Microscopy Contributions to Advances in Science and Technology*, Formatex Research Center, Badajoz, 2012, pp. 1013-1024.

133. Ueto, T.; Takata, N.; Muroyama, N.; Nedu, A.; Sasaki, A.; Tanida, S.; Terada, K.: Polymorphs and a Hydrate of Furosemide-Nicotinamide 1:1 Cocrystal, *Crystal Growth Design.*, 12 (2012) 485-494.
134. Li, J.; Bourne, S. A.; De Villiers, M. M.; Crider, A. M.; Caira, M. R.: Polymorphism of the Antitubercular Isoxyl, *Crystal Growth Design.*, 11 (2011) 4950-4957.
135. Rakotonirainy, M. S.; Bénéaud, O.; Vilmont, L. B.: Contribution to the characterization of foxing stains on printed books using infrared spectroscopy and scanning electron microscopy energy dispersive spectrometry, *Int. Biodeterior. Biodegradation*, 101 (2015) 1-7.
136. Flegler, S. L.; Heckman Jr., J. W.; Klomparens, K. L.: *Scanning and Transmission Electron Microscopy*, W. H. Freeman and Company, New York, 1993.
137. Stadtländer, C. T. K.-H.: *Scanning Electron Microscopy and Transmission Electron Microscopy of Mollicutes: Challenges and Opportunities*, in: Mendez-Vilas, A. and Diaz, J. (eds.), *Modern Research and Educational Topics in Microscopy*, Formatex Research Center, Badajoz, 2007, pp. 122-131.
138. Taylor, N.: *Energy Dispersive Spectroscopy*, John Wiley & Sons Ltd., Chichester, 2015.
139. Ayala, A. P.: Polymorphism in drugs investigated by low wavenumber Raman scattering, *Vibrational Spectroscopy*, 45 (2007) 112-116.
140. Simonea, E.; Saleemia, A. N.; Nagy, Z. K.: Application of quantitative Raman spectroscopy for the monitoring of polymorphic transformation in crystallization processes using a good calibration practice procedure, *Chem. Eng. Res. Des.*, 92 (2014) 594-611.
141. Zell, M. T. et al.: Investigation of Polymorphism in Aspartame and Neotame Using Solid-State NMR Spectroscopy, *Tetrahedron*, 56 (2000) 6603-6616.
142. Chyall, L. J.: Current Applications of X-Ray Powder Diffraction in the Pharmaceutical Industry, *American Pharmaceutical Review*, 15 (2012) 6.
143. Karjalainen, M.; Airaksinen, S.; Rantanen, J.; Aaltonen, J.; Yliruusi, J.: Characterization of polymorphic solid-state changes using variable temperature X-ray powder diffraction, *J. Pharm. Biomed. Anal.*, 39 (2005) 27-32.
144. Rastogi, S.K.; Zakrzewski, M.; Suryanarayanan, R.: Investigation of solid-state reactions using variable temperature X-ray powder diffractometry. I. Aspartame hemihydrate, *Pharm. Res.*, 18 (2001) 267-273.
145. Rollinger, J. M.; Burger, A.: Physico-chemical characterization of hydrated and anhydrous crystal forms of amlodipine besylate, *J. Therm. Anal. Calorim.*, 68 (2002) 361-372.
146. Ohannesian, L.; Streeter, A. J.: *Handbook of Pharmaceutical Analysis*, first ed., Marcel Dekker, New York, 2001.
147. Raza, K.; Kumar, P.; Ratan, S.; Malik, R.; Arora, S.: Polymorphism: The Phenomenon Affecting the Performance of Drugs, *SOJ Pharm Pharm Sci*, 1(2) [2004] 10.
148. Griesser, U. J.: The Importance of Solvates in: Hilfiker, R. (ed.) *Polymorphism: in the Pharmaceutical Industry*, Wiley-VCH, Weinheim, 2006., pp. 211-233.

149. Giron, D.: *Angewandte Chemische Thermodynamik und Thermoanalytic*, E. Marti and H. R. Oswald (Eds.), Birkhäuser Verlag, Basel, 1979, p. 227.
150. Sacchetti, M.: Thermodynamic analysis of DSC data for acetaminophen polymorphs, *J. Therm. Anal. Calorim.*, 63 (2001) 345-350.
151. Vitez, I. M.; Newman, A. W.; Davidovich, M.; Kiesnowski, C.: The evolution of hot-stage microscopy to aid solid-state characterizations of pharmaceutical solids, *Thermochimica Acta*, 324 (1998) 187-196.
152. Rodriguez, C. and Bugay, D. E.: Characterization of Pharmaceutical Solvates by Combined Thermogravimetric and Infrared Analysis, *J. Pharm. Sci.*, 86 (1997) 263-266.
153. Fang, Z.; Zhang, L.; Maob, S.; Rohani, S.; Ulrich, J.; Lu, J.: Solubility measurement and prediction of clopidogrel hydrogen sulfate polymorphs in isopropanol and ethyl acetate, *J. Chem. Thermodynamics*, 90 (2015) 71-78.
154. Giron, D.: Thermal analysis and calorimetric methods in the characterisation of polymorphs and solvates, *Thermochimica Acta*, 248 (1995) 1-59.
155. Német, Z.: *Gyógyszervegyületek szilárdfázisú analízise: Polimorfia vizsgálati módszerek a gyógyszeriparban*, PhD thesis, Budapest, 2009.
156. Matsuda, Y.; Kawaguchi, S.: Physicochemical characterization of oxyphenbutazone and solid-state stability of its amorphous form under various temperature and humidity conditions, *Chem. Pharm. Bull.*, 34 (1986) 1289-1298.
157. Brown, M. E. and Gallagher, P. K.: Recent Advances, Techniques and Applications, in: Brown, M. E. and Gallagher, P. K. (eds.) *Handbook of Thermal Analysis and Calorimetry*, Elsevier, Amsterdam, 2008, pp. 1-755.
158. Grillo, D.; Polla, G.; Vega, D.: Conformational Polymorphism on Imatinib Mesylate: Grinding Effects, *J. Pharm. Sci.*, 101 (2012) 541-551.
159. Rabel, S. R.; Jona, J. A.; Maurin, M. B.: Applications of modulated differential scanning calorimetry in preformulation studies, *J. Pharm. Biomed. Anal.*, 21 (1999) 339-345.
160. McGregor, C.; Saunders, M. H.; Buckton, G.; Saklatvala, R. D.: The use of high-speed differential scanning calorimetry (Hyper-DSCTM) to study the thermal properties of carbamazepine polymorphs, *Thermochim. Acta*, 417 (2004) 231-237.
161. Ford, J. L.; Mann, T. E.: Fast-scan DSC and its role in pharmaceutical physical form characterisation and selection, *Adv. Drug Deliv. Rev.*, 64 (2012) 422-430.
162. Muniz-Miranda, M.; Muniz-Miranda, F.; Caporali, S.: SERS and DFT study of copper surfaces coated with corrosion inhibitor, *Beilstein J. Nanotechnol.*, 5, (2014) 2489-2497.

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ANNEX

I.

Rapid communication

Applicability of sucrose laurate as surfactant in solid dispersions prepared by melt technology

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ABSTRACT

This study focused on an investigation of the applicability of sucrose laurate as surfactant in solid dispersions. Although this surfactant has a US Drug Master File, it has not been used so far in internal pharmaceutical products. High drug-loaded solid dispersion systems consisting of gemfibrozil as a model drug and PEG 6000 as a carrier, with or without sucrose laurate (D1216), were prepared by the melting method. Cytotoxicity studies on Caco-2 monolayer cells were also performed, in order to gain information on the applicability of D1216 in oral formulations. The results showed that the presence of the surface-active agent did not affect the solid-state characteristics of the model drug significantly. A markedly improved dissolution of gemfibrozil from the ternary solid dispersion systems was observed as compared with the binary solid dispersion systems. The optimum concentration range of the D1216 in the formulations was determined to be 5–10%. The effective final concentrations of D1216 in the dissolution experiments proved to be non-toxic towards Caco-2 cells. The results suggest the potential use of D1216 in innovative internal pharmaceutical formulations.

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The poor water solubility of drug substances and their low rates of dissolution in the aqueous gastrointestinal fluids often lead to insufficient bioavailability, and this remains a problem to the pharmaceutical industry. Solid dispersions of hydrophobic drugs in water-soluble carriers have attracted considerable interest as a means of improving dissolution behaviour, and hence enhancing bioavailability. Water-soluble carriers such as high-molecular-weight polyethylene glycols (PEGs) and polyvinylpyrrolidones (PVPs) have been most commonly used for solid dispersions (Bikiaris et al., 2005; Craig and Newton, 1991; Leuner and Dressman, 2000; Saharan et al., 2009; Serajuddin, 1999). The use of surfactants with solubilizing properties, such as polysorbates, poloxamers, Gelucires (polyethylene glycol glycerides), sodium lauryl sulfate or vitamin E TPGS have also attracted considerable interest recently (Dehghan and Jafar, 2006; Jagdale et al., 2010; Liu and Wang, 2007; Mura et al., 1999; Okonogi and Puttipipatkachorn, 2006; Owusu-Ababio et al., 1998; Sethia and Squillante, 2002; Vasconcelos et al., 2007). As described in the review by Vasconcelos et al. (2007), the third-generation solid dispersion systems contain a surfactant carrier, or a mixture of amorphous polymers and surfactants as carriers. These third-generation solid dispersions are intended to achieve the highest degree of bioavailability for poorly soluble drugs. The inclusion

of surfactants in the solid dispersions may help to avoid drug recrystallization and to stabilize the systems (Vasconcelos et al., 2007).

Sucrose esters (SEs) are widely used in the food and cosmetics industries, and there has recently been great interest in their applicability in different pharmaceutical fields. They are biodegradable, natural, non-ionic surface-active agents consisting of sucrose as hydrophilic moiety and fatty acids as lipophilic groups (Abd-Elbary et al., 2008; Csóka et al., 2007; Ganem Quintanar et al., 1998; Okamoto et al., 2005; Otomo, 2009; Ntawukuliyayo et al., 1993; Shibata et al., 2002).

In an earlier study we investigated, the structure and thermal behaviour of SE in order to predict their applicability in hot melt technology (Szűts et al., 2007). Our results revealed that SEs are semicrystalline carriers, with both amorphous and crystalline regions. During the preparation of solid dispersions, the drugs are built into the amorphous phases of the SEs (Szűts et al., 2008). In melt technology, mainly the lipophilic SEs may be suggested as carriers. They display characteristic melting, whereas SEs with high or moderate HLB values only soften during heating (Szűts et al., 2007). It has also been found that hydrophilic SEs exhibit gelling behaviour at body temperatures, which can influence the drug release (Szűts et al., 2010a,b). In view of these results, the applicability of hydrophilic SEs alone as carriers in hot melt technology is not suggested. Dispersion or dissolution of the drugs in the softened SEs is difficult, and a high amount of swelling SEs can reduce the rate of dissolution of a drug.

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The aim of the present study was to evaluate the applicability of hydrophilic sucrose ester as surfactant in third-generation solid dispersion systems together with a polymer. As carrier, the commonly used PEG 6000 was chosen, with which the hydrophilic (HLB=16) sucrose laurate showed the best miscibility among the evaluated sucrose esters (sucrose-stearate, -palmitate and -laurate). Although this surfactant has a US Drug Master File, it has so far not been used in internal pharmaceutical products. In this work, studies on the cytotoxicity of sucrose laurate on Caco-2 monolayer cells were also performed, in order to gain information on its availability in oral formulations.

Gemfibrozil (GEM), a poorly water-soluble ($29.1 \mu\text{g/ml}$ at $\text{pH}=6.2 \pm 0.1$) model drug, was supplied by TEVA (Hungary). PEG 6000, the carrier used in our experiments, was from Hungaropharma (Hungary). Sucrose laurate D1216 (HLB=16) was kindly provided by Harke Pharma GmbH (Germany).

During the sample production, 40% w/w of GEM was always applied. In the case of the binary systems, PEG 6000 was heated at 70°C in a sand bath and, after melting, the appropriate amount of GEM was added. In dispersions incorporating surfactant (1%, 5%, 10% or 15% w/w), D1216 was dissolved in the melted carrier prior to the addition of GEM. The molten mixture was stirred manually for 15 min, to achieve homogeneous dispersion of the drug. The melts were quickly cooled to -10°C in a freezer, after which the solidified samples were pulverized in a mortar and sieved to $200 \mu\text{m}$.

The physical states of the GEM in the different samples were evaluated by XRPD with a Miniflex II X-ray Diffractometer (Rigaku Co. Tokyo, Japan), where the tube anode was Cu with $K\alpha = 1.5405 \text{ \AA}$. Patterns were collected with a tube voltage of 30 kV and a tube current of 15 mA in step scan mode ($4^\circ/\text{min}$). The instrument was calibrated by using Si.

The release of the model drug was studied by using Pharmatest equipment (Hainburg, Germany) at a paddle speed of 100 rpm. 100 ml artificial enteric juice with a pH of 6.8 (± 0.05) at 37°C ($\pm 0.5^\circ\text{C}$) was used. The concentration of GEM was determined spectrophotometrically at 276 nm (Unicam UV/vis spectrophotometer). The dissolution experiments were conducted in triplicate.

The statistical test ANOVA was used to compare the results of dissolution data. The difference between samples was deemed statistically significant if the 95% confidence intervals for the means did not overlap ($p < 0.05$).

The effect of D1216 on living cells was tested by using the human colon carcinoma cell line CaCo-2 (ATCC, USA), a model of the intestinal epithelium (Breemen and Li, 2005). Cells were grown in Eagle's minimal essential medium (MEM, Invitrogen) supplemented with 15% foetal bovine serum (Lonza, Switzerland) and 1% Na-pyruvate (Sigma, Hungary). Confluent monolayers were obtained in 96-well plates (Orange Scientific, Belgium) 3 days after cell seeding. For toxicity experiments, Dulbecco's Modified Eagle's medium (DMEM) without phenol red was used as assay medium. Two different cytotoxicity tests were performed. The lactate dehydrogenase (LDH) assay detects cell damage and death by measuring the release of the cytoplasmic enzyme LDH from cells due to plasma membrane disruption. The LDH levels in culture medium were determined with a commercially available kit (Cytotoxicity Detection Kit LDH, Roche, Switzerland). An increase in the number of dead or membrane-damaged cells results in an increase in LDH activity in the cell-free culture supernatant. Cytotoxicity was calculated as a percentage of the total LDH release from cells treated with 1% Triton X-100 as detergent. The MTT test measures cell viability, because only living and metabolically active cells can convert the yellow tetrazolium salt (MTT, Sigma M5655) into insoluble purple formazan crystals. The extent of dye conversion was determined spectrophotometrically by measuring the absorbance at 570 nm. In the MTT assay, a

Fig. 1. X-ray power diffraction data of pure materials and solid dispersion systems.

decrease in dye reduction correlates to the cell damage. Viability was calculated as a percentage of the number of untreated control cells. All experiments were repeated at least three times; the number of parallel wells for each treatment and time point varied between 4 and 8.

An earlier study revealed that the hydrophilic sucrose stearate and sucrose palmitate do not melt during heating, but only soften (Szűts et al., 2007). In consequence of this thermal behaviour, the distribution of a drug in their melts is difficult, and can result in an inhomogeneous product. Hence, in the present study, the applicability of sucrose laurate as a surfactant was examined in a third-generation solid dispersion system. The solid dispersions were prepared by the melting method, containing GEM as model drug and PEG 6000 as carrier, with or without D1216 as surfactant. In the ternary solid dispersion systems, when D1216 was used up to 15%, a homogeneous melt could be formed.

Intact GEM and PEG 6000 displayed identical sharp XRPD peaks at various values of 2θ , while the X-ray pattern of D1216 exhibited an amorphous, broad halo (Fig. 1). In order to determine the crystallinity degree of drug in solid dispersions, the intensity of the most characteristic peak of GEM (intensity: 7767 at $2\theta = 12.06$) was evaluated in the various systems. The XRPD pattern of GEM binary solid dispersions demonstrated the diffraction peak of the crystalline drug. This suggested that GEM existed in the crystalline state in the solid dispersion system. However, the intensity of the peaks of crystalline GEM in the solid dispersions was significantly lower than that of the intact drug, indicating a lower degree of crystallinity of GEM in the binary solid dispersion system (inten-

Fig. 2. Dissolution curves of GEM and solid dispersion systems containing various concentrations (0, 1, 5, 10 and 15%) of D1216 as surfactant.

Fig. 3. Cytotoxicity measurements on various concentrations of D1216 on Caco-2 epithelial cells by LDH release after treatment for 1 h with D1216, and MTT dye conversion following treatment for 4 h (means $\pm$ SD, $n = 8-4$).

sity: 2500 at $2\theta = 11.52$). As the drug was highly loaded into the solid dispersion, some of the GEM molecules were molecularly dispersed, and a higher amount of GEM existed in the crystalline state. The XRPD patterns of the ternary solid dispersions were similar to those of the binary system (intensities of the most characteristic peak of ternary systems: 2070 at $2\theta = 11.64$ (GEM-PEG-D1216 40-55-5), 1950 at $2\theta = 11.56$ (GEM-PEG-D1216 40-50-10) and 2452 at $2\theta = 11.52$ (GEM-PEG-D1216 40-45-15) (Fig. 1). The incorporation of D1216 up to 15% had no effect on the XRPD pattern of GEM in the solid dispersion system.

Fig. 2 illustrates that the D1216 in samples resulted in significantly higher GEM release than that of started GEM. For the 1% D1216-containing solid dispersion, the drug release was similar ($p > 0.05$) that to form the binary solid dispersion system, whereas 5% SE resulted in significantly faster release. In this case, 90% of the GEM had dissolved after 10 min. With increasing content of D1216, the dissolution rate increased further. 100% GEM release could be attained after 10 min on the use of 10% D1216 (Fig. 2). Increase of the D1216 concentration from 10% to 15% did not result in significantly faster release. Accordingly, 5–10% D1216 seems to be optimum for solid dispersions of GEM.

PEGs have been used extensively as carriers for solid dispersions due to their favourable solution properties, low melting points and low toxicity. Thanks to these characteristics, they are approved by the FDA for internal consumption.

Besides improving dissolution, surfactants can also enhance absorption, thereby increasing the bioavailability of poorly soluble drugs (Deli, 2009). However, surfactants may be cytotoxic, which can reduce their applicability in oral formulations (Dimitrijevic et al., 2000; Ekelund et al., 2005; Kiss et al., 2010). In this study, therefore, cytotoxicity measurements were made on the human intestinal epithelial cell line Caco-2 in order to determine the maximum non-toxic concentration of D1216 as absorption enhancer. The cytotoxicity of various concentrations of D1216 in LDH tests is shown in Fig. 3. The concentration of D1216 that caused no toxicity after 1 h was below 200 $\mu\text{g/ml}$. In the MTT studies, the duration of treatment was 4 h, and significant toxicity was observed when the D1216 concentration exceeded 100 $\mu\text{g/ml}$ (Fig. 3). Above 600 $\mu\text{g/ml}$ D1216, high toxicity occurred, resulting in the death of Caco-2 cells (Fig. 3).

Our dissolution studies shown, that applying 5–10% D1216 was optimum for GEM solid dispersions. In these formulations, the concentrations of D1216 in the dissolution media were 83.3 $\mu\text{g/ml}$ (5% D1216) and 166.7 $\mu\text{g/ml}$ (10% D1216), proved to be non-toxic towards Caco-2 cells.

The cytotoxicity studies demonstrated similarly as with other surfactants, that, when the internal applicability of sucrose laurate is under consideration, the possible risk of the local effect of an increased concentration in the microenvironment of the

gastrointestinal tract must be taken into account. It should be noted that the SEs are widely used in different food products, and their acceptable daily intake was set as 40 mg/kg/day. Sucrose laurate was not considered in that evaluation, but the European Food Safety Authority (EFSA) recently pointed out that the current specifications should be changed to include sucrose laurate (EFSA, 2010).

It can be concluded that the applicability of sucrose laurate in third-generation solid dispersions prepared by melt technology may be regarded as a good technique with which to accelerate the dissolution of poorly soluble drugs such as GEM. The presence of 1–15% surface-active agent did not appear to affect the solid-state characteristics of GEM significantly. The in vitro dissolution studies shown, that applying 5–10% D1216 was optimum to improve GEM release from solid dispersions. In these formulations, the concentrations of sucrose laurate in the dissolution media were 83.3 $\mu\text{g/ml}$ (5% D1216) and 166.7 $\mu\text{g/ml}$ (10% D1216), proved to be non-toxic towards Caco-2 cells.

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References

- Abd-Elbary, A., El-laithy, H.M., Tadros, M.I., 2008. Sucrose stearate-based proniosome derived niosomes for the nebulisable delivery of cromolyn sodium. *Int. J. Pharm.* 357, 189–198.
- Bikiaris, D., Papageorgiou, G.Z., Stergiou, A., Pavlidou, E., Karavas, E., Kanaze, F., Georgarakis, M., 2005. Physicochemical studies on solid dispersions of poorly water-soluble drugs. Evaluation of capabilities and limitations of thermal analysis techniques. *Thermochim. Acta* 439, 58–67.
- Breemen, R.B., Li, Y., 2005. Caco-2 permeability assays to measure drug absorption. *Expert Opin. Drug Metab. Toxicol.* 1, 175–185.
- Craig, D.Q.M., Newton, J.M., 1991. Characterisation of polyethylene glycol solid dispersions using differential scanning calorimetry and solution calorimetry. *Int. J. Pharm.* 76, 17–24.
- Csóka, G., Marton, S., Zelko, R., Otomo, N., Antal, I., 2007. Application of sucrose fatty acid esters in transdermal therapeutic systems. *Eur. J. Pharm. Biopharm.* 65, 233–237.
- Dehghan, M.H.G., Jafar, M., 2006. Improving dissolution of meloxicam using solid dispersions. *Iran. J. Pharm. Res.* 4, 231–238.
- Deli, M.A., 2009. Potential use of tight junction modulators to reversibly open membranous barriers and improve drug delivery. *Biochim. Biophys. Acta* 1788, 892–910.
- Dimitrijevic, D., Shaw, A.J., Florence, A.T., 2000. Effects of some non-ionic surfactants on transepithelial permeability in Caco-2 cells. *J. Pharm. Pharmacol.* 52, 157–162.
- EFSA Panel on Food Additives Nutrient Sources added to Food (ANS), 2010. Scientific opinion on the safety of sucrose esters of fatty acids prepared from vinyl esters of fatty acids and on the extension of use of sucrose esters of fatty acids in flavourings. *EFSA J.* 8, 1512.

- Ekelund, K., Osth, K., Pählstorp, C., Björk, E., Ulvenlund, S., Johansson, F., 2005. Correlation between epithelial toxicity and surfactant structure as derived from the effects of polyethyleneoxide surfactants on caco-2 cell monolayers and pig nasal mucosa. *J. Pharm. Sci.* 94, 730–744.
- Ganem Quintanar, A., Quintanar-Guerrero, D., Falson-Rieg, F., Buri, P., 1998. Ex vivo oral mucosal permeation of lidocaine hydrochloride with sucrose fatty acid esters as absorption enhancers. *Int. J. Pharm.* 173, 203–210.
- Jagdale, S.C., Kuchekar, B.S., Chabukswar, A.R., Musale, V.P., Jadhao, M.A., 2010. Preparation and in vitro evaluation of Allopurinol-Gelucire 50/13 solid dispersions. *Int. J. Adv. Pharm. Sci.* 1, 60–67.
- Kiss, T., Fenyvesi, F., Bácskay, I., Váradi, J., Fenyvesi, E., Iványi, R., Szente, L., Tószaki, A., Vecsernyés, M., 2010. Evaluation of cytotoxicity of β -cyclodextrin derivatives: evidence for the role of cholesterol extraction. *Eur. J. Pharm. Sci.* 40, 376–380.
- Leuner, C., Dressman, J., 2000. Improving drug solubility for oral delivery using solid dispersions. *Eur. J. Pharm. Biopharm.* 50, 47–60.
- Liu, L., Wang, X., 2007. Improved Dissolution of Oleanolic Acid with Ternary Solid Dispersions. *AAPS PharmSciTech.* 8, Article 113.
- Mura, P., Faucci, M.T., Manderioli, A., Bramanti, G., Parrini, P., 1999. Thermal behavior and dissolution properties of naproxen from binary and ternary solid dispersions. *Drug Dev. Ind. Pharm.* 25, 257–264.
- Ntawukulilyayo, J.D., Bouckaert, S., Remon, J.P., 1993. Enhancement of dissolution rate of nifedipine using sucrose ester coprecipitates. *Int. J. Pharm.* 93, 209–214.
- Okamoto, H., Takashi, S., Kazumi, D., 2005. Effect of sucrose fatty acid esters on transdermal permeation of lidocaine and ketoprofen. *Biol. Pharm. Bull.* 28, 1689–1694.
- Okonogi, S., Puttipatkhachorn, S., 2006. Dissolution Improvement of High Drug-loaded Solid Dispersion. *AAPS PharmSciTech.* 7, Article 52.
- Otomo, N., 2009. Basic properties of sucrose fatty acid esters and their applications. In: Hayes, D.G., Kitamoto, D., Solaiman, D.K.Y., Ashby, R.D. (Eds.), *Biobased Surfactants and Detergents: Synthesis, Properties, and Applications*. AOCS Press, Urbana, Illinois, pp. 275–298.
- Owusu-Ababio, G., Ebube, N.K., Reams, R., Habib, M., 1998. Comparative dissolution studies for mefenamic acid-polyethylene glycol solid dispersion systems and tablets. *Pharm. Dev. Technol.* 3, 405–412.
- Saharan, V.A., Kukkar, V., Kataria, M., Gera, M., Choudhury, P.K., 2009. Dissolution enhancement of drugs. Part I: Technologies and effect of carriers. *Int. J. Health Res.* 2, 107–124.
- Serajuddin, A.T.M., 1999. Solid dispersion of poorly water-soluble drugs: early promises subsequent problems, and recent breakthroughs. *J. Pharm. Sci.* 88, 1058–1066.
- Sethia, S., Squillante, E., 2002. Physicochemical characterization of solid dispersions of carbamazepine formulated by supercritical carbon dioxide and conventional solvent evaporation method. *J. Pharm. Sci.* 91, 1948–1957.
- Shibata, D., Shimada, Y., Yonezawa, Y., Sunada, H., Otomo, N., Kasahara, K., 2002. Application and evaluation of sucrose fatty acid esters as lubricants in the production of pharmaceuticals. *J. Pharm. Sci. Technol.* 62, 133–145.
- Szűts, A., Pallagi, E., Regdon, G., Aigner, Z., Szabó-Révész, P., 2007. Study of thermal behaviour of sugar esters. *Int. J. Pharm.* 336, 199–207.
- Szűts, A., Makai, Zs., Rajkó, R., Szabó-Révész, P., 2008. Study of the effects of drugs on the structures of sucrose esters and the effects of solid-state interactions on drug release. *J. Pharm. Biomed. Anal.* 48, 1136–1142.
- Szűts, A., Budai-Szűcs, M., Erős, I., Otomo, N., Szabó-Révész, P., 2010a. Study of gel-forming properties of sucrose esters for thermosensitive drug delivery systems. *Int. J. Pharm.* 383, 132–137.
- Szűts, A., Budai-Szűcs, M., Erős, I., Ambrus, R., Otomo, N., Szabó-Révész, P., 2010b. Study of thermo-sensitive gel-forming properties of sucrose stearates. *J. Excipients Food Chem.* 1, 13–20.
- Vasconcelos, T., Sarmento, B., Costa, P., 2007. Solid dispersions as strategy to improve oral bioavailability of poor water soluble drugs. *Drug Discov. Today* 12, 1068–1075.

II.

Polymorph screening of an active material

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ABSTRACT

Polymorph screening is currently one of the most important tasks for innovators and for generic companies from both pharmaceutical and intellectual property rights aspects. The different polymorphs have different physicochemical properties, such as the crystal polymorph-dependent solubility which influences the bioavailability.

A former drug candidate obtained from Sanofi Pharmaceutical Company (Hungary) was investigated to explore its polymorphism, to distinguish the morphologies generated by analytical examinations and to investigate their relative stabilities. An Avantium Crystal 16 automatic laboratory reactor system was used for the polymorph studies and the studies of their dissolution. Eight polymorphs were obtained by crystallization and transformation methods then characterized by XRPD, DSC, and Raman spectroscopy, scanning electron microscopy, and light microscopy. All the morphologies could be stored in solid without any form transformation for a long time (2 years investigated). According to the first relative stability results, Form I, III, IVa, V, VI, VII are unambiguously metastable forms. Form II and IVb have similar thermodynamic stabilities, that were higher than those of the other polymorphs.

A special dissolution medium was developed in which the eight polymorphs showed clear differences in the rate of dissolution.

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1. Introduction

Polymorphism, the ability of an element or compound to crystallize in more than one distinct crystal species [1], is a major problem that has been of considerable importance in the pharmaceutical industry in the development of new drug candidates [2,3]. The different polymorphs exhibit different physical and physicochemical properties, such as habit, colour, density, melting point, solubility, dissolution rate, etc. [4]. These differences may affect the pharmaceutical processing, the stability of the drug product, the bioavailability and the toxicity, and thereby the therapeutic efficacy of the drug substance [5]. Furthermore, each crystal phase can be protected by patents [6], which is very important for the innovator. During the polymorphism screening process, it is necessary to investigate the relative stability, because mostly thermodynamically stable forms are used in the drug products [7].

The most common polymorph screening methods are crystallization from melt, vapour, and solutions through cooling or evaporating the solvent, addition of an antisolvent to the solution [8], or slurring the solid active pharmaceutical ingredient for an extended period of time at different temperatures [9].

The outcome of a crystallization can be affected by factors such as the solvent (viscosity, polarity), the rate of creating supersaturation (cooling, adding or evaporation rate), or initial solution concentration [10]. Other crystallization conditions, e.g. the rate of stirring, different mechanical processes (e.g. ultrasound effect) or different additives, may also influence the final crystal polymorph obtained.

The most common methods for the characterization of the various polymorphs are powder X-ray diffractometry (XRPD), single-crystal X-ray diffractometry (SC-XRD), differential scanning calorimetry (DSC), optical and electron microscopy, infrared (IR), near-infrared (NIR), Raman, and more recently solid-state nuclear magnetic resonance spectroscopy (ssNMR) [11–16].

In case of polymorphism the relative stability and the transformation conditions of the different polymorphs must be investigated [17]. Polymorphic transformations between crystalline modifications of an active pharmaceutical ingredient in, e.g. crystallization, heating/cooling studies and milling have been already characterized by various solid-state analytical methods [18]. An Avantium Crystal 16 automatic laboratory reactor system was used for such investigations. This medium-throughput polymorph and salt screening technology performs 16 parallel crystallization experiments, and provides an estimate of solubility by using turbidity measurements [19]. With this reactor system, the solubility and supersolubility curves can also be determined [20].

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Fig. 1. Chemical structure of the former drug candidate.

The model compound of this study was a former drug candidate obtained from Sanofi Pharmaceutical Company. Here, a description of the polymorphism of a drug candidate is presented without using additives during the generating process. The polymorphs were generated by crystallization methods (cooling, evaporation) and heating transformation, furthermore the modifications obtained were distinguished by means of various analytical examinations. For the dissolution study, a special dissolution medium was developed in which the different polymorphs could be distinguished.

2. Experimental

2.1. Materials

The model drug used was 3-[2-({1-(2-cyclohexylethyl)-5-(2,5-dimethoxy-4-methylphenyl)-1H-1,2,4-triazol-3-yl}amino)carbonyl]-6-methoxy-4,5-dimethyl-1H-indol-1-yl]propanoic acid (Fig. 1). The crystallization of the various polymorphic forms was achieved with different organic solvents of analytical grade: acetonitrile, methanol, 96% ethanol, abs. ethanol, isopropanol, acetone, 2-butanone, toluene (Merck, Budapest, Hungary), dichloromethane, ethyl acetate, butyl acetate, chloroform, 1,4-dioxane (Reanal, Budapest, Hungary), and tetrahydrofuran (Aldrich, Budapest, Hungary).

2.1.1. Preparation of polymorphs by crystallization

Different techniques were used for the preparation of the polymorphs (Table 1):

Crystallization by shock cooling: The raw material was dissolved at the boiling point of the solvent to give a saturated solution which was then diluted with a small amount of the solvent. The hot solution was filtered into a vial immersed into crashed ice.

Crystallization by slow cooling: The same process of dissolution was used, but the filtered, hot solution was cooled down slowly ($-3^{\circ}\text{C}/\text{h}$).

Crystallization by slow evaporation: The products were the dried residues of the clear filtrates of the samples prepared by shock cooling.

2.1.2. Preparation of polymorphs by heating transformation

Through heating processes at various temperatures for different periods of time, the polymorphs generated by crystallization could be transformed to other forms.

2.2. Investigation methods

2.2.1. Identification of crystalline forms of samples

2.2.1.1. Powder X-ray diffractometry. XRPD spectra were recorded with a BRUKER D8 Advance diffractometer (Bruker AXS GmbH, Karlsruhe, Germany) system with $\text{Cu K}\alpha_1$ radiation ($\lambda = 1.5406 \text{ \AA}$) over the interval $2.5\text{--}40^{\circ}/2\theta$. The measurement conditions were as follows: target, Cu; filter, Ni; voltage, 40 kV; current, 40 mA; time constant, 0.1 s; angular step 0.016° . Detector: NaI (TI) scintillation detector.

2.2.1.2. Differential scanning calorimetry. DSC curves were obtained by a Mettler Toledo DSC27HP apparatus (Mettler-Toledo AG, Greifensee, Switzerland) under the following conditions: sample weight: about 2–3 mg; sample holder: aluminum crucible (40 μL) with lid; nitrogen flow rate: 100–150 mL/min; heating rate: $10^{\circ}\text{C}/\text{min}$ from 25°C up to 250°C . In every case, samples were held at 25°C for 10 min before recording.

2.2.1.3. Raman spectroscopy. Raman spectra were recorded at room temperature by using a Bruker SENTERRA Dispersive Raman Microscope (Bruker Optik GmbH, Ettlingen, Germany) equipped with Nd-YAG (532 nm) and diode (785 nm) excitation lasers and a cooled CCD detector. The system was fitted with a motorized XYZ sample stage. The equipment was controlled by OPUS 6.5 software (Bruker Optik GmbH, Ettlingen, Germany). Samples were analyzed on glass slides.

Following a parameter optimization process, the samples were measured by Nd-YAG (532 nm) laser at a laser power of 10 mW. An integration time of 5 s, and 3 scans were used for each measurement, and spectra were collected over the Raman shift range of $3500\text{--}60 \text{ cm}^{-1}$ at a resolution of $3\text{--}5 \text{ cm}^{-1}$. The microscope magnification was $200\times$ and a certain crystal was selected for analysis with

Table 1

The techniques and conditions used for preparation of the polymorphs.

Form	Method	Solvent	Conditions
I	Crystallization	96%, abs. ethanol	Shock cooling
II	Heating	–	$160^{\circ}\text{C}/6 \text{ h}$ (from Form I)
III	Crystallization	Isopropanol, 2-butanone, butylacetate, ethylacetate, toluene	Shock cooling
IVa	Crystallization	Methanol	Shock cooling
IVb	Heating	–	$205^{\circ}\text{C}/6 \text{ h}$ (from Form IVa)
V	Crystallization	Chloroform	Shock cooling, slow cooling, slow evaporation
VI	Crystallization	Acetone, methanol, dichloromethane, 1,4-dioxane	Shock cooling, slow cooling, slow evaporation
VII	Crystallization	Acetonitrile	Shock cooling

a 50 μm $\times$ 1000 μm grating. Single Raman spectra of the individual crystals were collected in each case.

2.2.1.4. Scanning electron microscopy. The morphology of the particles was examined by SEM (Hitachi S4700, Hitachi Scientific Ltd., Tokyo, Japan). A sputter coating apparatus (Bio-Rad SC 502, VG Microtech, Uckfield, United Kingdom) was applied to induce electric conductivity on the surface of the samples. The air pressure was 1.3–13.0 mPa.

2.2.1.5. Particle size analysis. The particle size distribution of the drug candidate was measured by LEICA Image Processing and Analysis System (LEICA Q500MC, LEICA Cambridge Ltd., Cambridge, United Kingdom). The particles were described in terms of their length, breadth, surface area, perimeter and *roundness* which is a shape factor giving a minimum value of unity for a circle. This is calculated from the ratio of perimeter squared to area. The adjustment factor of 1.064 corrects the perimeter for the effect of the corners produced by the digitization of the image.

$$\text{Roundness} = \frac{\text{Perimeter}^2}{4 \cdot \pi \cdot \text{Area} \cdot 1.064}$$

2.2.2. Physicochemical properties of the forms

2.2.2.1. Dissolution examinations. Dissolution was studied by a modified paddle dissolution apparatus (Pharmatest, Hainburg, Germany) at a paddle speed of 100 rpm. 100 mL of dissolution medium was placed in a 37 °C (± 0.5 °C) bath and the examination was performed for 2 h. Because of the poor solubility of the model drug, neither gastric acid nor intestinal fluid could be used alone. Therefore, an appropriate dissolution medium had to be developed.

The preliminary examination of the model compound showed that its solubility depends on pH: at low pH (gastric acid) is worse than at neutral pH (intestinal fluid), therefore intestinal fluid (pH=6.8) was chosen, but the dissolution even in intestinal fluid was not up to 100%. As all the 8 polymorphs dissolved well in 96% ethanol, the optimum mixture of 96% ethanol: intestinal fluid (pH=6.8) was found of a volumetric ratio of 3:7. The concentration of each polymorph solution was determined spectrophotometrically at 324 nm (Unicam UV/vis spectrophotometer, Unicam Limited, Cambridge, United Kingdom). The dissolution experiments were conducted in triplicate and standard deviation was also calculated.

2.3. Relative stability examinations

An Avantium Crystal 16 automatic laboratory reactor system was used to investigate the relative stabilities of the different polymorphs. The organic solvents for the relative stability examination were chosen regarding to the solubility properties of each polymorph (very high, medium, and low solubility). First 96% ethanol was applied for different periods of time at various temperatures.

3. Results and discussion

3.1. XRPD

XRPD is always the definitive method for the identification of polymorphs [4]. The XRPD patterns of the 8 polymorphs are shown in Fig. 2. The characteristic diffraction peaks are presented in Table 2. The data in Table 2 indicate that the characteristic peaks are situated between 4 and 26 2 θ degree. All the 8 polymorphs (with the exception of Forms IVa and IVb) exhibit clear XRPD differences, but these two forms can be well distinguished by other methods, though Form IVa and IVb were very similar to each other.

Table 2

The characteristic diffraction peaks of the 8 polymorphs.

	<i>d</i> (Å)	2 θ (°)	Intensity (counts)	<i>I</i> / <i>I</i> ₁ (%)
Form I	20.054	4.403	10.30	100.0
	10.388	8.505	0.61	5.9
	6.64	13.319	0.92	8.9
	5.926	14.937	0.89	8.7
	4.995	17.744	1.00	9.7
	4.156	21.360	6.71	65.0
	3.3983	22.300	2.58	25.0
	3.463	25.707	1.01	9.8
	2.605	34.405	0.49	4.7
	2.429	36.983	0.40	3.9
Form II	18.386	4.802	24.50	100.0
	8.212	10.756	7.99	32.6
	6.023	14.696	4.10	16.7
	5.694	15.549	4.98	20.4
	5.085	17.428	4.32	17.6
	4.587	19.336	5.51	22.5
	4.387	20.225	6.25	25.5
	4.117	21.570	4.85	19.8
	3.684	24.139	14.40	58.7
	3.562	24.977	3.35	13.7
Form III	10.715	8.245	16.30	100.0
	9.579	9.224	0.71	4.4
	9.125	9.685	1.56	9.6
	8.419	10.498	1.59	9.8
	8.043	10.992	0.78	4.8
	4.207	21.099	2.09	12.9
	4.038	21.995	2.49	15.3
	3.636	24.465	0.98	6.0
	3.572	24.903	1.47	9.0
	2.908	30.719	0.66	4.0
Form IVa	12.531	7.048	2.28	34.6
	10.005	8.831	2.09	31.8
	8.304	10.645	1.93	29.3
	6.276	14.100	3.03	46.0
	5.329	16.622	2.27	34.5
	4.816	18.408	3.16	48.0
	4.448	19.947	1.95	29.7
	4.166	21.309	6.59	100.0
	3.617	24.592	3.05	46.3
	3.486	25.533	2.51	38.2
Form IVb	12.543	7.042	72.40	53.1
	10.041	8.799	94.50	69.3
	8.286	10.669	81.20	59.6
	6.304	14.037	81.90	60.1
	5.342	16.582	82.10	60.3
	5.186	17.083	78.50	57.6
	4.444	19.962	72.70	53.4
	4.175	21.263	136.00	100.0
	4.120	21.550	72.70	53.4
	3.627	24.521	62.30	45.7
Form V	19.665	4.490	0.41	5.1
	16.458	5.365	8.01	100.0
Form VI	10.818	8.167	2.56	32.4
	8.163	10.830	0.63	8.0
	5.766	15.355	0.70	8.9
	5.419	16.343	7.88	100.0
	4.144	21.423	1.67	21.2
	3.819	23.267	4.68	59.4
	3.743	23.750	0.68	8.6
	3.627	24.595	3.28	41.6
	3.121	28.582	0.58	7.4
	2.406	37.350	0.59	7.4
Form VII	17.004	5.193	14.30	100.0
	10.306	8.573	8.61	60.3
	9.715	9.096	8.50	59.5
	8.833	10.006	2.81	19.7
	6.208	14.255	4.92	34.4
	5.154	17.191	2.63	18.4
	4.892	18.121	2.25	15.8
	4.164	21.321	7.67	53.7
	3.876	22.925	3.95	27.7
	3.446	25.835	2.46	17.2

Fig. 2. The XRPD patterns of the 8 polymorphs.

3.2. DSC

The DSC curves and the relevant data of the 8 polymorphs are shown in Fig. 3 and Table 3. The 8 forms give different DSC patterns, except for Form I and II which display a single endothermic peak at about 232 °C (under the DSC conditions, Form I is converted to Form II). Without melting, the transformation of Form I–II could be observed by hot-stage microscopy.

Forms III, V, VI, and VII have multiple peaks (endotherms and exotherm) which illustrate transformation by heating via melting. The presence of solvated morphologies or hydrated ones were excluded by TG, H^1 -NMR and elemental analysis. The drug substance has several solvates, but in this study it was dealt only with “real” polymorphs. According to the humidity XRPD results, the polymorphs were stable in the interval 20–80% RH, no hydrated morphologies occurred. These four polymorphs furnish a common

Fig. 3. The DSC curves of the 8 polymorphs.

peak at around 232 °C which corresponds to Form II. The melting and recrystallization transformation could also be observed by hot-stage microscopy. Therefore five out of the eight polymorphs transformed to Form II by heating via melting (monotropy) or without it (enantiotropy).

For Form IVa, 2 endothermic peaks are observed at 219 and 227 °C, whereas Form IVb gives only 1 endothermic peak at 227 °C, which indicates the transformation caused by heating from Form IVa to IVb. The possibility of Form IVa being a solvated or hydrated form of Form IVb was also excluded in the same way as presented above.

3.3. Raman spectroscopy

Raman spectroscopy is an analytical technique that is widely applied for the chemical and physico-chemical characterization of solid compounds as it is a non-destructive method and needs only minimal sample preparation. Furthermore, in contrast with IR spectroscopy, it is not disturbed by the presence of water. Raman spectra are a highly specific method, serving as the fingerprints of the molecules, but the material itself or its impurities may cause background fluorescence which makes the analysis difficult or sometimes impossible.

Table 3
The relevant DSC data on the 8 polymorphs.

Form	T_1 (°C)	ΔH (J/mol)	T_2 (°C)	ΔH (J/mol)	T_3 (°C)	ΔH (J/mol)	T_4 (°C)	ΔH (J/mol)
I	231.4	−30485.96	–	–	–	–	–	–
II	232.0	−57561.94	–	–	–	–	–	–
III	214.3	−44675.68	219.1	+4089.51	231.8	−15184.30	–	–
IVa	218.7	−36793.19	226.6	−12144.97	–	–	–	–
IVb	227.3	−30615.69	–	–	–	–	–	–
V	130.9	−6603.75	174.8	+28904.52	233.4	−48610.75	–	–
VI	171.4	−15388.15	178.3	+18458.37	231.0	−54238.45	–	–
VII	184.0	−16320.96	188.3	+13053.06	215.4	−605.40	231.4	−22411.97

Fig. 4. The Raman spectra of the 8 polymorphs.

Raman spectroscopy generally has better spectral selectivity (for the distinction of polymorphic forms including the amorphous state) as the symmetric stretching linked to the carbon skeleton is more expressed than IR spectroscopy has.

There are clear differences at the ranges 3200–2800 cm^{-1} and 1800–1000 cm^{-1} (Figs. 4 and 5). The spectra of Form I and Form IVb demonstrate low intensities comparing to the other forms (5000 and 1200 a.u. vs. 20,000–40,000 a.u.). Furthermore Form II and Form IVa exhibit slight fluorescence behaviour.

Between 3200 cm^{-1} and 2700 cm^{-1} each of the polymorphs except for Forms I and IVb gives a single peak at 2843 cm^{-1} . Form VII has another peak at 3010 cm^{-1} . Form III can be easily distinguished by the single peaks at 3042 and 2966 cm^{-1} . Form VII has 3 discriminative peaks between 3100 cm^{-1} and 2950 cm^{-1} (Fig. 4).

Between 1800 cm^{-1} and 900 cm^{-1} further differences can be observed. Form V has unique peaks at 1677 cm^{-1} , 1476 cm^{-1} and 1036 cm^{-1} while Form VII also has singular peaks at 1694 cm^{-1} , 1542 cm^{-1} , 1325 cm^{-1} and 1247 cm^{-1} . Minor differences are to be

Fig. 5. The Raman spectra of the 8 polymorphs.

seen at about 1550 cm^{-1} . Characteristic peaks can also be found in the range 1400–900 cm^{-1} (Fig. 5).

3.4. SEM

The SEM pictures (Figs. 6 and 7) reveal that the crystals of the polymorphs present differences in size, morphology and surface. Form III obtained by shock-cooling in isopropanol, 2-butanon, or ethyl acetate gave mostly irregular trapezoid crystals with a smooth surface around 50 μm . Form I (shock cooling in ethanol) resulted in long needle-shaped crystals. When Form I was heated to 160 $^{\circ}\text{C}$, the transformation led to a new morphology. Form II contained prismatic crystals in size range of 25–60 μm . Form V crystals obtained by shock cooling in chloroform are around 25 μm , with a nearly ditrigonal morphology.

The morphology of Form IVa obtained by shock cooling in methanol and Form IVb obtained by heating of Form IVa were

Fig. 6. The SEM pictures of Forms I, II, III and V.

Fig. 7. The SEM pictures of Forms IVa, IVb, VI and VII.

very similar, involving slender ditrigonal and ditetragonal prisms. The crystals of Form IVa were smaller than those of Form IVb. The crystals of Form VI and Form VII were very irregular and varied in size.

3.5. Particle size analysis

About 400 particles were analyzed by light microscopy. Table 4 presents the results. Most of the particles had sizes in the range 10–25 μm . The smallest particles were those of Form IVa, and the largest were those of Form VI. Form I and II gave very similar average results. Form V exhibited the smallest roundness value which explains the best dissolution profile. Form II and IVb were prepared by heating transforming one polymorph into another, and there was no significant difference in particle size.

3.6. Dissolution examinations

As it is known, particle size has effect on solubility and dissolution rate, therefore unified particle size is required. Despite

of it, in our case there were clear differences in the dissolution between the different polymorphs which was not related to the particle size. In the special dissolution medium, the rate of dissolution could be well distinguished (Fig. 8). The fastest dissolution and the largest amount of dissolved compound were seen for Form V, whereas Form III displayed the slowest dissolution, but the lowest amount dissolved belonged to Form IVa. At Forms IVa and IVb a “burst effect” was observed at the beginning of the curve. This is in connection with the small particle size of these 2 polymorphs. After the 2 h dissolution examination the undissolved remaining of the 8 polymorphs were checked by XRPD and no change in the polymorphic forms could be detected in this special dissolution medium.

3.7. Relative stability examination

Investigating the individual polymorphs in 96% ethanol, the transformation of Forms I, V, VI, and VII could be observed to Form III even at room temperature, while Forms II, III, IVa and IVb remained unchanged during the investigated period (130 days).

Table 4
The average results of the particle size analysis.

Form		Length (μm)	Width (μm)	Perimeter (μm)	Area (μm^2)	Roundness
I	Average	22.7	12.7	68.7	214.9	1.99
	SD $\pm$	13.7	6.6	45.3	300.5	1.13
II	Average	22.5	13.1	77.8	203.1	2.41
	SD $\pm$	12.5	7.0	69.2	201.6	2.18
III	Average	20.4	12.2	62.7	231.8	1.77
	SD $\pm$	13.8	8.7	46.2	336.7	0.75
IVa	Average	10.4	4.6	27.6	33.2	1.85
	SD $\pm$	4.1	1.7	11.2	21.8	0.61
IVb	Average	17.8	6.3	44.3	78.6	2.10
	SD $\pm$	11.4	3.3	28.5	84.4	1.00
V	Average	20.3	14.3	61.9	259.9	1.38
	SD $\pm$	11.8	8.3	40.2	334.7	0.43
VI	Average	109.8	63.5	354.2	5348.4	2.12
	SD $\pm$	62.2	40.2	279.0	5970.1	1.44
VII	Average	31.2	20.0	106.3	476.8	2.23
	SD $\pm$	17.9	11.5	88.9	541.7	1.97

Fig. 8. The extents of dissolution of the 8 polymorphs.

Forms III and IVa transformed to Form IVb at elevated temperature (50 °C and 70 °C). In 96% ethanol Form II and Form IVb did not transform at all.

The preliminary results of the relative stability examinations demonstrated that the stability of Forms II and IVb are similar and higher than those of the other polymorphs.

4. Conclusion

Eight polymorphs for the compound were generated by a polymorph-screening protocol and distinguished by several analytical methods (XRPD, DSC, Raman-spectroscopy, and SEM). Their relative stability was investigated by an Avantium Crystal 16 automatic laboratory reactor system. A special dissolution medium for the screening of our polymorphic modifications was developed.

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References

- [1] J. Halebian, W. McCrone, Pharmaceutical applications of polymorphism, *J. Pharm. Sci.* 58 (1969) 911–929.
- [2] H.G. Brittain, *Polymorphism in Pharmaceutical Solids*, Marcel Dekker, New York, 1999.
- [3] J. Bernstein, *Polymorphism in Molecular Crystals*, Clarendon Press, Oxford, 2002.
- [4] Y. Li, P.S. Chow, R.B.H. Tan, Quantification of polymorphic impurity in an enantiotropic polymorph system using differential scanning calorimetry, X-ray powder diffraction and Raman spectroscopy, *Int. J. Pharm.* 415 (2011) 110–118.
- [5] J.Th.H. Van Eupen, R. Westheim, M.A. Deij, H. Meekes, P. Bennema, E. Vlieg, The solubility behaviour and thermodynamic relations of the three forms of Venlafaxine free base, *Int. J. Pharm.* 368 (2009) 146–153.
- [6] I. Karabas, M.G. Orkoul, C.G. Orkoul, Analysis and stability of polymorphs in tablets: the case of Risperidone, *Talanta* 71 (2007) 1382–1386.
- [7] K. Jarring, T. Larsson, B. Stensland, I. Ymén, Thermodynamic stability and crystal structures for polymorphs and solvates of formoterol fumarate, *J. Pharm. Sci.* 95 (2006) 1144–1161.
- [8] C. Sun, Solid-state properties and crystallization behavior of PHA-739521 polymorphs, *Int. J. Pharm.* 319 (2006) 114–120.
- [9] A.M. Campeta, B.P. Chekal, Y.A. Abramov, P.A. Meenan, M.J. Henson, B. Shi, R.A. Singer, K.R. Horspool, Development of a targeted polymorph screening approach for a complex polymorphic and highly solvating API, *J. Pharm. Sci.* 99 (2010) 3874–3886.
- [10] A. Getsoian, R.M. Lodaya, A.C. Blackburn, One-solvent polymorph screen of carbamazepine, *Int. J. Pharm.* 348 (2008) 3–9.
- [11] J. Aaltonen, M. Alles, S. Mirza, V. Koradia, K. Gordon, C.J. Rantanen, Solid form screening—a review, *Eur. J. Pharm. Biopharm.* 71 (2009) 23–37.
- [12] D.E. Bugay, Characterization of the solid-state: spectroscopic techniques, *Adv. Drug Deliv. Rev.* 48 (2001) 43–65.
- [13] G.A. Stephenson, R.A. Forbes, S.M. Reutzel-Edens, Characterization of the solid state: quantitative issues, *Adv. Drug Deliv. Rev.* 48 (2001) 67–90.
- [14] A.W. Newman, S.R. Byrn, Solid-state analysis of the active pharmaceutical ingredient in drug products, *Drug Dis. Today* 8 (2003) 898–905.
- [15] B. Shan, V.K. Kakumanu, A.K. Bansal, Analytical techniques for quantification of amorphous/crystalline phases in pharmaceutical solids, *J. Pharm. Sci.* 95 (2006) 1641–1665.
- [16] J. Aaltonen, K.C. Gordon, C.J. Strachan, T. Rades, Perspectives in the use of spectroscopy to characterise pharmaceutical solids, *Int. J. Pharm.* 364 (2008) 159–169.
- [17] A.G. Lord, BPC's and cGMP's, *Pharm. Eng.* 8 (1988) 30–35.
- [18] A. Chieng, T. Rades, J. Aaltonen, An overview of recent studies on the analysis of pharmaceutical polymorphs, *J. Pharm. Biomed. Anal.* 55 (2011) 618–644.
- [19] M. Birch, S.J. Fussell, P.D. Higginson, N. McDowall, I. Marziano, Towards a PAT-based strategy for crystallization development, *Org. Process Res. Dev.* 9 (2005) 360–364.
- [20] M.P. Feth, N. Nagel, B. Baumgartner, M. Bröckelmann, D. Rigal, B. Otto, M. Spitzenberg, M. Schulz, B. Becker, F. Fischer, C. Petzoldt, Challenges in the development of hydrate phases as active pharmaceutical ingredients—an example, *Eur. J. Pharm. Sci.* 42 (2011) 116–129.

III.

Analysis of the polymorph changes of a drug candidate

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ABSTRACT

The effects of solvents, temperature and humidity on the stability of a former drug candidate obtained from Sanofi (Hungary) were examined by a slurry equilibration method, variable temperature and humidity X-ray powder diffractometry (VT/VH-XRPD) and differential scanning calorimetry (DSC). The VH-XRPD study showed that all 8 polymorphic forms of this material were stable in the interval 20–80 RH%. The VT-XRPD measurements indicated that all the polymorphs except Form II underwent changes in the range 30–200 °C. The stable form was Form II, though Form IVb had almost the same stability. The investigation demonstrated that VT-XRPD is a very useful *in situ* method for relative stability studies.

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1. Introduction

During pharmaceutical production, materials go through many processes, such as compression, granulation, grinding, drying [1]. Polymorphs have different lattice energies, and forms with higher energy tend to transform into forms with lower energy [2]. The storage conditions, such as humidity or temperature, affect the stability of the crystal forms [3–7]. Characterization of the polymorphs generated and clarification of the thermodynamic stability relations are important during the polymorphism screening process [2].

It was reported earlier that the rate of transformation of a form in suspension depended on the temperature and its solubility in the suspending medium used [2]. If polymorphs do not display distinct differences in their enthalpies of fusion or/and no phase transition peak is found in the DSC curve [8], the enthalpies and melting points determined by DSC are not sufficient for a detailed evaluation of transitions and thermodynamic stabilities. In that case, the solubility data of the forms are especially important in the investigation of the phase transitions of the various polymorphs [2].

Research, product usage and patent protection of pharmaceuticals can involve high importance being placed on polymorph screening and the use of powder diffraction [9]. X-ray powder diffractometry (XRPD) provides the most direct and definitive

identification of polymorphs and can offer a means for the quantitative analysis of polymorphic mixtures [10]. XRPD analysis based on diffraction peak angles and the corresponding intensities is often preferred for the quantification of polymorphic forms [11]. More accurate information can be obtained by XRPD when the temperature and/or humidity are variable.

Variable-temperature XRPD (VT-XRPD) is a technique where XRPD experiments are carried out at different temperatures [1]. Through VT-XRPD analysis, complex pharmaceutical solid-state reactions, including crystal structure transformations, can be characterized *in situ* [12,13]. The VT and variable-humidity (VH) sample chambers of the XRPD instrument allow the crystal form changes associated with the changing conditions to be followed [14].

Other important methods of detecting polymorph transitions are calorimetric methods such as differential scanning calorimetry (DSC) or modulated-temperature differential scanning calorimetry (MTDSC). DSC is widely used for polymorphism studies, because the various crystalline polymorphs frequently differ in their heats of fusion [2]. The occurrence of multiple peaks when a form is heated can indicate a polymorph transformation [15]. The heating rate may have a great influence on the kinetics and the resolution of peaks in DSC curves [16]. At a comparatively high heating rate (e.g. 100 °C min^{−1}), the kinetics of the melting transition is changed as if there were not enough time for recrystallization of the higher melting form [17]. With fast-scan DSC, the melting of the metastable polymorph can be distinguished from any subsequent recrystallization because the later event is moved to a higher temperature and provides separation of the events [18].

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Fig. 1. Chemical structure of the former drug candidate.

MTDSC differs from conventional DSC in that the sample is subjected to a more complex heating programme, incorporating a sinusoidal temperature modulation accompanied by an underlying linear heating jump ramp. While the DSC method measures the total heat flow, MTDSC can distinguish reversible (heat capacity component) and non-reversible (kinetic component) heat flows too [19].

In a previous study, a former drug candidate obtained from Sanofi (Hungary) was examined and 8 polymorphs (Forms I, II, III, IVa, IVb, V, VI and VII) were generated and distinguished by different analytical methods. At the early beginning of the polymorph screening of this former drug candidate, it was not possible to decide if Form IVa and IVb are a mixture of two polymorphs or they are pure polymorphs, but the further analytical investigations could prove that they are separate forms [20].

The aim of the current study was to use three different methods of investigation (isothermal suspension equilibration testing, XRPD and DSC) to study the polymorph changes of the model compound and to rank the polymorphs generated in terms of their relative stability.

2. Experimental

2.1. Materials

The model compound used was 3-[2-({1-(2-cyclohexylethyl)-5-(2,5-dimethoxy-4-methylphenyl)-1H-1,2,4-triazol-3-yl}amino)carbonyl]-6-methoxy-4,5-dimethyl-1H-indol-1-yl]propanoic acid (Fig. 1). The crystallization of the various polymorphic forms was achieved with different organic solvents of analytical grade: acetonitrile, methanol, 96% ethanol, absolute ethanol, isopropanol, acetone, 2-butanone, toluene (Merck, Budapest, Hungary), dichloromethane, ethyl acetate, *n*-butylacetate, chloroform, 1,4-dioxane (Reanal, Budapest, Hungary), and tetrahydrofuran (Aldrich, Budapest, Hungary). The polymorphs generated were stored under normal conditions.

2.2. Methods

2.2.1. Isothermal suspension equilibration

An automatic laboratory reactor system (Avantium Crystal 16, Amsterdam, The Netherlands) was used to investigate the relative stabilities of the different polymorphs in suspension. Crystal 16 is a multiple reactor system working as a parallel crystallizer on a 1 ml volume scale, equipped with an online turbidity probe. The organic solvents for the relative stability examination were chosen with regard to the solubility properties of each polymorph. The different polymorphs were stirring in suspension in 96% ethanol as a “moderately good” solvent and in silicone oil as a heat-transferring medium. 100 mg samples of the polymorphs were examined in 1 ml of 96% ethanol or silicone oil for a maximum of 130 days at room temperature, 50 or 70 °C (in the case of ethanol) or at 200 °C (in case of silicone oil). Samples of the suspensions were taken out from time to time with a glass pipette, filtered and placed on a Si low-background smooth-surfaced sample holder, and the crystals obtained were measured by XRPD.

2.2.2. VH/VT-XRPD

VH/VT-XRPD patterns were recorded with a Bruker D8 Advance diffractometer (Bruker AXS GmbH, Karlsruhe, Germany) system with Cu K α 1 radiation ($\lambda = 1.5406 \text{ \AA}$) in the interval $2.5\text{--}40^\circ/2\theta$. The diffractometer was equipped with a hot-humidity chamber (MRI Physikalische Geräte GmbH, Karlsruhe, Germany) controlled by an Ansyc Sycos H-Hot (Analytische Systeme und Komponenten GmbH, Karlsruhe, Germany) and a Vântec 1 line detector (Bruker AXS GmbH, Karlsruhe, Germany), which indicated the phase transitions of the polymorphs directly in the diffractometer chamber. The measurement conditions: target, Cu; filter, Ni; voltage, 40 kV; current, 40 mA; time constant, 0.1 s; angular step, 0.007° .

The parameters of the VH-XRPD investigations: humidity between 20 and 80 RH% in 10 RH% increments, and repeated measurement at 20 RH%. The temperature was 30 °C. The VT-XRPD studies were carried out between 30 and 230 °C, in increments of 5 °C. After the series of measurements, the samples were cooled back to 30 °C and measurements were repeated immediately after cooling and one day later.

2.2.3. DSC

The DSC analysis was carried out with a Mettler Toledo STAR[®] thermal analysis system, version 9.30 DSC 821[®] (Mettler-Toledo AG, Greifensee, Switzerland), at a linear heating rate of 1, 10 or 30 °C min⁻¹, with nitrogen as carrier gas (100 ml min⁻¹). The sample weight was in the range 2–5 mg and examinations were performed in the temperature interval 25–250 °C, in a sealed 40 μ l aluminium crucible having two leaks in the lid. In every case, samples were held at 25 °C for 10 min before measurements.

The MTDSC parameters: temperature interval, 25–250 °C; heating rate: 10 °C min⁻¹; amplitude: 0.5 or 1 °C; period: 0.5 or 1 min.

3. Results and discussion

3.1. Isothermal suspension equilibration

When the ethanol suspensions were slurried at room temperature, Forms I, V, VI and VII turned into Form III, whereas Forms II, III, IVa and IVb did not undergo any change within 130 days. At 50 °C, Form I was transformed to Form III, and Form III was transformed to Form IVb. The other forms investigated (Form II, IVa and IVb) remained unchanged. At 70 °C, Form I turned into Form II, Form III into Form IVb, and Form IVa to Form IVb, while Forms II and IVb did not change during the investigated period.

When the polymorphs of the model compound were slurried in silicone oil at 200 °C, Forms I and III turned into Form II and Form IVa into Form IVb. The other forms were not investigated. No interchange was observed between Forms II and IVb.

3.2. VH-XRPD

VH-XRPD analysis on each of the 8 polymorphs clearly showed that all of the polymorphs were stable in the range 20–80 RH% and at 20 RH% in the repeated measurements. As an example, the results of the investigation of Form I are presented in a two-dimensional figure (Fig. 2), where the intensities of the reflections are proportional to the intensity of the grey colour.

Neither new reflection peaks nor intensity differences were detected during the examinations of these 8 polymorphs when the RH in the measuring chamber was either increased or decreased.

3.3. VT-XRPD

The *in situ* VT-XRPD investigations yielded some interesting results as concerns the polymorph transformation screening process. The intensities of the diffraction peaks of Form I decreased

Fig. 2. 2D VH-XRPD diffractogram of Form I in the interval 20–80 RH% and repeated at 20 RH%.

continuously during heating. The transformation of Form I started at about 155 °C and finished at about 190 °C (Fig. 3). After cooling back, the intensities of the diffraction peaks were slightly increased as compared with those of the sample measured at 200 °C. The result of the transformation was Form II. The volume change caused by the temperature change resulted in slight shifts in the reflection peaks. A similar phenomenon was observed in the examination of each polymorph.

The temperature increase did not cause any phase transition of Form II; this polymorphic form remained stable until melting 230 °C and after cooling back to 20 °C (Fig. 4). The VT-XRPD investigation indicated that this form was the stable one, and this was confirmed by the DSC studies.

The phase transition of Form III started at about 215 °C. After recooling, the new low-intensity reflection peaks measured at 230 °C were increased significantly; Form II was identified. The shifting of the smaller-intensity reflection peaks at 2θ

values of about 16–17 and the larger-intensity peaks at 2θ values of around 25 started at 160 °C, which indicated the earlier onset of this phase transition with some content of Form I (Fig. 5).

Increase of the temperature did not cause any changes in the Form IVa and IVb polymorphs up to 225 °C. Further increase of temperature resulted in the melting of these samples, and after cooling back to 30 °C the samples became amorphous. Repeated measurements on the next day verified the persistence of the amorphous form. Because of the limitations of the VT-XRPD instrument, it was not able to achieve a sufficiently high temperature for the transformation of Form IVa to Form IVb.

During the heating of Form V, the disappearance of diffraction peaks was observed at about 145 °C. Thermomicroscopic studies confirmed that this phenomenon was caused by the melting process. Crystallization of the melt started at about 150 °C. The diffractogram of this new form (Intermediate I) was different from

Fig. 3. 2D VT-XRPD diffractogram of Form I in the interval 30–200 °C.

Fig. 4. 2D VT-XRPD diffractogram of Form II in the interval 30–230 °C.

Fig. 5. 2D VT-XRPD diffractogram of Form III in the interval 30–230 °C.

that of any other polymorph. During further heating of the material, a phase-transition process started at about 190 °C, resulting in a second new form (Intermediate II), and Form II finally crystallized at about 220 °C. The diffraction peaks of the material cooled down to 20 °C those of pure Form II (Figs. 6 and 7).

During the heating of Form VI two phase-transition processes were detected, at onset temperatures of about 175 °C and 190 °C. The result of the first process was the same as the previously detected Intermediate I, while the second series of peaks were those of Form II (Fig. 8).

Finally, similarly to Form I, Form VII was transformed to Form II. The onset temperature of the phase transition was about 190 °C.

Intermediates I and II could not be prepared in this investigation. These two forms are instable forms; all attempts to isolate them resulted in the stable Form II.

3.4. DSC

DSC investigations of all the polymorphic forms were performed at several heating rates. The results of these studies

Fig. 6. 2D VT-XRPD diffractogram of Form V in the interval 30–230 °C.

Fig. 7. VT-XRPD diffractograms of Form V at several temperatures.

Fig. 8. 2D VT-XRPD diffractogram of Form VI in the interval 30–230 °C.

Fig. 9. DSC curves of Form I at different heating rates.

Fig. 10. DSC curves of Form III at different heating rates.

Fig. 11. DSC curves of Form V at different heating rates.

Table 1
The relevant DSC data of the 8 polymorphs.

Form	T_{onset} (°C)	$\pm$ s.d.	ΔH (J g ⁻¹)	$\pm$ s.d.
I	229.9	0.7	-49.0	0.2
II	230.8	0.6	-93.6	0.5
III	212.8	0.5	-70.5	0.5
IVa	215.7	0.1	-56.7	0.7
IVb	218.9	0.3	-56.2	0.2
V	121.3	0.3	-11.0	0.6
VI	170.0	0.1	-43.9	0.1
VII	178.7	0.4	-57.9	0.2

confirmed the findings of the XRPD investigations. The DSC studies carried out at different heating rates led to different results. Both the low (1 °C min⁻¹) and the high (30 °C min⁻¹) heating rate studies provided additional information on the phase-transition processes.

The polymorph phase-transition peak of Form I could not be detected at low heating rate. However, a heating rate of 30 °C min⁻¹ caused a small exothermic peak at an onset temperature of 190 °C, which indicated that Form I was transformed into the more stable Form II. It was also observed that a higher heating rate generally caused the peaks to shift towards higher temperatures (Fig. 9). A similar phenomenon was observed for Form VII.

Depending on the heating rate, DSC curves of Form II contained one endothermic peak at about 231–235 °C, which corresponded to the melting point of the most stable Form II.

The phase-transition process of Form III to Form II was seen in a lower heating rate DSC curve (an endothermic peak at 213 °C and a subsequent exothermic peak at 214–215 °C). These processes could not be distinguished from each other at the highest heating rate. The slow phase-transformation process between 160 and 215 °C described in the XRPD investigations was not detected in the DSC curves (Fig. 10).

In the DSC curves of Form V the endothermic peak of melting could be identified at about 132–146 °C. At heating rates of 1 and 10 °C min⁻¹, only the first phase-transition process could be observed, which was shifted towards higher temperatures on increase of the heating rate. The two phase-transition processes could be seen only in the DSC curve at higher heating rate (Fig. 11).

Fig. 12. Flowchart of phase-transition processes.

The phase-transition process of Form VI was detected in the DSC curve at the heating rate of 10 °C min⁻¹. The endothermic and exothermic pair at about 178 and 186 °C corresponded to the appearance of the Intermediate I form. The phase transition could not be detected at a heating rate of 1 °C min⁻¹, while the subprocesses could not be separated from each other at a heating rate of 30 °C min⁻¹.

The MTDSC investigations did not provide more information on the relative stability of the polymorphs, and these results are therefore not presented here.

The enthalpies of Forms III, IVa, VI and VII were only estimated, due to the inability to obtain a single melting endotherm. The enthalpy of the melting endotherm for Form II was found to be the highest, and that for Form V was the lowest (Table 1). Form II was not transformed to Form IVb or vice versa.

4. Conclusions

The relative stabilities of the polymorphs of a former-drug candidate were investigated by an isothermal suspension equilibration

method, variable-humidity and temperature X-ray powder diffraction and differential scanning calorimetry. The summarized results of the studies are presented in a flowchart (Fig. 12).

The isothermal suspension equilibration and VT-XRPD methods led to different phase-transformation processes for some polymorphic forms. The phase-transition processes were confirmed by DSC studies carried out at different heating rates. The VT-XRPD investigations demonstrated the formation of two new unstable intermediate forms (from Forms V and VI). Forms IVa and IVb resulted in amorphous materials. Variation of the relative humidity had no influence on the polymorphic transformations. It was concluded that Forms I, III, IVa, V, VI and VII are metastable forms, while Form IVb has almost the same stability as that of Form II because the transformation of Form IVb to Form II (and *vice versa*) has not been achieved to date. The enthalpy data, the isothermal suspension equilibration investigations and the XRPD results confirmed that the thermodynamically stable polymorph is Form II. It may be noted that the VT-XRPD method is a very useful technique for the analysis of phase-transition processes. The application of different heating rates during the DSC tests is recommended in order to obtain appropriate information about the details of phase-transformation processes.

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References

- [1] M. Karjalainen, S. Airaksinen, J. Rantanen, J. Aaltonen, J. Yliruusi, Characterization of polymorphic solid-state changes using variable temperature X-ray powder diffraction, *J. Pharm. Biomed. Anal.* 39 (2005) 27–32.
- [2] H.G. Brittain, *Polymorphism in Pharmaceutical Solids*, vols. 1–18, Marcel Dekker, New York, 1999, pp. 251–256.
- [3] Y. Matsuda, S. Kawaguchi, Physicochemical characterization of oxyphenbutazone and solid-state stability of its amorphous form under various temperature and humidity conditions, *Chem. Pharm. Bull.* 34 (1986) 1289–1298.
- [4] Y. Matsuda, E. Tatsumi, Physicochemical characterization of furosemide modifications, *Int. J. Pharm.* 60 (1990) 11–26.
- [5] M. Otsuka, M. Onoe, Y. Matsuda, Physicochemical stability of phenobarbital polymorphs at various levels of humidity and temperature, *Pharm. Res.* 10 (1993) 577–582.
- [6] G.Z. Zhang, C. Gu, M.T. Zell, R.T. Burkhardt, E.J. Munson, D.W. Grant, Crystallization and transitions of sulfamerazine polymorphs, *J. Pharm. Sci.* 91 (2002) 1089–1100.
- [7] X.C. Tang, M.J. Pikal, L.S. Taylor, The effect of temperature on hydrogen bonding in crystalline and amorphous phases in dihydropyridine calcium channel blockers, *Pharm. Res.* 19 (2002) 484–490.
- [8] C.Q. Yang, Z.W. Zhang, Y.L. Zeng, J. Wang, Structures and characterization of m-nisoldipine polymorphs, *Cryst. Eng. Comm.* 14 (2012) 2589–2594.
- [9] A. Clearfield, J.H. Reibenspies, N. Bhuvanesh, *Principles and Applications of Powder Diffraction*, Wiley-Blackwell, Chichester, 2008, pp. 41.
- [10] R. Helmy, G.X. Zhou, Y.W. Chen, L. Crocker, T. Wang, R.M. Wenslow Jr., A. Vailaya, Characterization and quantitation of aprepitant drug substance polymorphs by attenuated total reflectance Fourier transform infrared spectroscopy, *Anal. Chem.* 75 (2003) 605–611.
- [11] M.M. de Villiers, R.J. Terblanche, W. Liebenberg, E. Swanepoel, T.G. Dekker, M. Song, Variable-temperature X-ray powder diffraction analysis of the crystal transformation of the pharmaceutically preferred polymorph C of mefenbamazole, *J. Pharm. Biomed. Anal.* 38 (2005) 435–441.
- [12] S.K. Rastogi, M. Zakrzewski, R. Suryanarayanan, Investigation of solid-state reactions using variable temperature X-ray powder diffractometry. I. Aspartame hemihydrate, *Pharm. Res.* 18 (2001) 267–273.
- [13] J.M. Rollinger, A. Burger, Physico-chemical characterization of hydrated and anhydrous crystal forms of amlodipine besylate, *J. Therm. Anal. Calorim.* 68 (2002) 361–372.
- [14] L. Ohannesian, A.J. Streeter, *Handbook of Pharmaceutical Analysis*, first ed., Marcel Dekker, New York, 2001.
- [15] R. Bottom, The role of modulated temperature differential scanning calorimetry in the characterisation of a drug molecule exhibiting polymorphic and glass forming tendencies, *Int. J. Pharm.* 192 (1999) 47–53.
- [16] D. Giron, Thermal analysis and calorimetric methods in the characterization of polymorphs and solvates, *Thermochim. Acta* 248 (1995) 1–59.
- [17] C. McGregor, M.H. Saunders, G. Buckton, R.D. Saklatvala, The use of high-speed differential scanning calorimetry (Hyper-DSC/TM) to study the thermal properties of carbamazepine polymorphs, *Thermochim. Acta* 417 (2004) 231–237.
- [18] J.L. Ford, T.E. Mann, Fast-scan DSC and its role in pharmaceutical physical form characterisation and selection, *Adv. Drug Deliv. Rev.* 64 (2012) 422–430.
- [19] S.R. Rabel, J.A. Jona, M.B. Maurin, Applications of modulated differential scanning calorimetry in preformulation studies, *J. Pharm. Biomed. Anal.* 21 (1999) 339–345.
- [20] P. Láng, V. Kiss, R. Ambrus, G. Farkas, P. Szabó-Révész, A. Zoltán, E. Várkonyi, Polymorph screening of an active material, *J. Pharm. Biomed. Anal.* 84 (2013) 177–183.

3. sz. melléklet: nyilatkozat az értekezés eredetiségéről

NYILATKOZAT SAJÁT MUNKÁRÓL

Név: dr. Láng Péter

A doktori értekezés címe: Polymorph screening of a former drug candidate

Én, *dr. Láng Péter* teljes felelősségem tudatában kijelentem, hogy a Szegedi Tudományegyetem Gyógyszertudományok Doktori Iskolában elkészített doktori (Ph.D.) disszertációm saját kutatási eredményeimre alapulnak. Kutatómunkám, eredményeim publikálása, valamint disszertációm megírása során a Magyar Tudományos Akadémia Tudományetikai Kódexében lefektetett alapelvek és ajánlások szerint jártam el.

Szeged, 2016. február 1.