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PhD Thesis

Amorphization of a crystalline active agent with the aim of pharmaceutical technological formulation

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LIST OF PUBLICATIONS

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ABSTRACTS

1. **Laczkovich O.**, Szabóné Révész P.: Kristályos anyagok amorfizálása gyógyszerformulálási céllal: *Műszaki Kémiai Napok*, Veszprém, 2003. április 8-10. (oral presentation)

2. **Laczkovich O.**, Szabóné Révész P., Erős I.: Hatóanyagok amorf formájának gyógyszer-technológiai előállítási lehetőségei: Congressus Pharmaceuticus Hungaricus XII, Budapest, 2003. május 8-10. (poster presentation)

3. **O. Laczkovich**, P. Szabó-Révész, M. Hasznos-Nezdei, I. Erős: Amorphization of crystalline substances with the aim of pharmaceutical technological formulation: International Meeting on Pharmaceutics, Biopharmaceutics and Pharmaceutical Technology 2004, Nürnberg, Germany, 15-18 March 2004 (poster presentation)

4. P. Szabó-Révész, **O. Laczkovich**, R. Ambrus, A. Szűts, Z. Aigner: Protocols for amorphization of crystalline solids through the application of pharmaceutical technological processes: 2nd BBBB Conference on Pharmaceutical Sciences, Tallin-Tartu, Estonia, 13-15 September 2007 (oral presentation)

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10. Cs. Mártha, **O. Jójárt-Laczkovich**, P. Szabó-Révész: Amorphization of co-ground clopidogrel hydrogensulphate: 8th World Meeting on Pharmaceutics, Biopharmaceutics and Pharmaceutical Technology, Istambul, Turkey, 19-22 March 2012 (poster presentation)

CONTENTS

1 INTRODUCTION

The two forms of solids are the crystalline form and the amorphous form (Cui, 2007). Solid materials are usually processed in their crystalline form, but application of the amorphous form is increasing. Glassy materials are used in many industrial fields glassy materials are used such as the plastics industry, the textile industry, the food industry, and for the production of semiconductors, ceramics and optical glasses, and naturally in the pharmaceutical industry.

In pharmaceutical formulations, most drug materials are processed in their crystalline form. This is a thermodynamically stable state that exhibits both short-range and long-range order (Hancock & Zografi, 1997; Aaltonen et al., 2009). Unlike a crystalline solid, an amorphous solid has no long-range order of molecular packing, so the molecules are conformationally flexible (Yu, 2001). The application of an active pharmaceutical ingredient (API) in amorphous form is increasingly common in the development of pharmaceutical solid formulations, with all its risks and benefits (Craig et al., 1999; Pokharkar et al., 2006).

What are the most important advantages of the application of the amorphous drugs? Amorphous forms of APIs have many useful properties. Among the most important ones are a higher dissolution rate and a sometimes higher water solubility relative to that of the crystalline form (Rodríguez-Spong et al.,2004; Zhang et al, 2004; Takeuchi et al., 2005; Hancock & Parks, 2000) as there is no lattice energy, which is a thermodynamic barrier to dissolution (Singhal & Curatolo, 2004).

It must be mentioned that there are disadvantages to the use of this form. Amorphous solids generally have lower stability than the corresponding crystals because of the higher energy level (Chadha et al., 2005, Craig et al., 1999, Pokharkar et al., 2006). Crystallization inhibitors therefore have to be used in most cases in amorphous pharmaceutical technological formulations. A wide range of auxiliary agents are available to stabilize this form and to prepare a suitable glassy dosage form.

In this way, two possibilities to achieve an amorphous phase can be differentiated: the first is when an amorphous material is prepared alone, as a pure glassy drug; and the second is when auxiliary agents (crystallization inhibitors) are used to prepare the amorphous systems.

The latter preparation methods result in solid dispersions. From the aspect of stability, this latter possibility is more appropriate and more applicable industrially.

The pharmaceutical industry is highly interested in amorphous formulations because amorphization techniques are very innovative, thanks to the advances in the analytical methods. The detection of amorphous forms is currently a widely investigated field of pharmaceutical technology, as concern both deliberate amorphization and when an unwanted glassy form appears spontaneously during formulation or storage.

In connection with the amorphous form in pharmaceutical technology, the most important reviews have been written by in this scientific field. They are Craig, Hancock, Zografi, Kerč, Srčič, Yu etc. Kerč and Srčič published the first results relating to the amorphous form of APIs in 1995, in Thermochimia Acta. The first significant review article, connected with amorphous materials in pharmaceutical technology (Yu, 2001), discussed preparation methods, characterization techniques and possibilities for the stabilization of glassy drugs. From a Hungarian aspect, our team first reported the advantages of amorphization in 2003 and used different methods in the industrial research and development work.

2 AIMS

The primary aim of this study was to establish the literature background of pharmaceutical amorphization. We wanted to know what methods are available to produce this special solid form, and how amorphous materials can be investigated and characterized with different analytical techniques.

The secondary aim was to investigate clopidogrel hydrogensulfate (CLP) as model drug from the aspect of pharmaceutical amorphization. The steps of this work were as follows:

- Characterization of the glassy property of CLP: determination of the investigation methods that can be used to classify CLP according to its glass formability (a poor or a good glass-former).
- Choice of a preparation method which results in pure amorphous CLP without use of an auxiliary agent.
- Investigation of the stability of pure amorphous CLP because of its tendency to undergo recrystallization during storage.
- Identification of a suitable recrystallization inhibitor and determination of its amount which can stabilize the amorphous form of CLP.
- Use of the amorphized product in a scaling-up process.
- Development of tablets as final dosage form that is appropriately stable as concern the recrystallization of CLP.
- Devising a protocol of amorphization in general, as a practical consideration.

It should be mentioned that the experimental part of this thesis was carried out in 2002-2004. In that period, the pharmaceutical industry was greatly in the amorphization of APIs. The amorphous form remains important nowadays but the approach has changed appreciably. Deliberate amorphization is still of great interest industrially, but in the scientific field, a new issue has arisen and has been subject to considerable development. This is when an amorphous form arises spontaneously during the pharmaceutical formulation or during storage. This can give rise to different properties which may cause problems in the processing technology or in the application of drugs.

3 LITERATURE BACKGROUND

3.1 Amorphization in pharmaceutical technology

The amorphous or glassy form is one of the two solid subphases; the other is the crystalline form (Cui, 2007). In pharmaceutical technology, this solid form is well known and widely studied because of its advantageous properties (Forster et al., 2002; Franks, 2002). The applications of amorphization can be divided into three groups:

- the amorphization of inorganic crystalline materials (Ziewiec et al., 2009);
- **the amorphization of organic materials consisting of small molecules (most APIs can be classified in this group)** (Panchagnula & Bhardwaj, 2008); and
- the amorphization of large polymer molecules (Casas et al., 2009).

The present work is concerned with the second point, using a model API for amorphization.

Amorphization can be applied in pharmaceutical technology for four reasons:

- to increase the dissolution rate and solubility of a poorly water-soluble API (Hancock & Parks, 1999; Leuner & Dressman, 2000; Forster et al., 2001; Kinoshita et al., 2002),
- to protect active agents from a polymorphous transformation (Zhang et al., 2004),
- to revise the processibility of the corresponding crystalline drug (Bozic et al., 2008), and
- to take out a new patent relating to the amorphous form of a given API (Lifshitz et al., 2004).

3.1.1 Increase of dissolution rate and solubility

In practice, many APIs are applied that display poor solubility in water. New drug materials are nowadays rarely classified into Biopharmaceutics Classification System groups II and IV. Because of their poor solubility in water, these active agents do not have sufficient bioavailability (Singhal & Curatolo, 2004).

This point is also important in the development of generic formulations. When an original drug product contains a crystalline API, with a better dissolution profile than that of the amorphous formulation, a new generic formulation method may be started.

It is a great problem that many API candidates exhibit poor solubility in water. In preclinical studies, when a high concentration is needed in the serum, amorphization may increase the solubility of an API.

There are examples, where the goal of amorphization was to achieve better solubility. Indomethacin (Hédoux et al., 2009), piroxicam (Tantishaiyakul et al., 1999) and curcumin (Paradkar et al., 2004) were amorphized with this aim. It should be mentioned that there is an example of amorphization not increasing but decreasing the solubility of a drug material. This API is rifampicin (Panchagnula & Bhardwaj, 2008).

3.1.2 Protection from polymorphic transformation

Numerous applied APIs have several polymorphic forms, with different physical and/or physico-chemical properties. These forms can be interconverted during the formulation process. In this way, the API can display changes in solubility, melting point, processibility and (not least) physiological effects. Such a polymorphic conversion may be prevented through amorphization (Singhal & Curatolo, 2004). For example, carbamazepine has different polymorphic forms, with different dissolution properties. However, the amorphous form of this API can also be prepared.

3.1.3 Improvement of processibility

Most of the work in this field deals with the polymorphic transformation of crystalline drug substances, but several studies have paid attention to the mechanical properties of non-crystalline APIs (Hancock et al., 2002). When the processing of crystalline drug substances lead runs into difficulties, it is possible that the amorphous form can be treated easily. In many cases, for example, the compressibility of the amorphous form is better than that of the crystalline form (Trasi et al., 2011). In Trasi et al. investigated the dehydration of glucose, during which the amorphous content of the glucose increased and hence the mechanical properties (e.g. the compressibility) of glucose improved. Bozic et al. (2008) investigated a macrolide antibiotic, which is a perfect example from the literature of how the compressibility of a drug can be revised by amorphization. In their work, the capping problem during tablet compression was reduced by amorphization.

3.1.4 Taking out a new patent

When the aim of amorphization is a new patent relating to the amorphous form, economic aspects are also involved. Patents concerning amorphous formulations are taken out with a wide range of aims. At the end of the 1990s and the beginning of the 2000s, years numerous inventors dealt with the compressibility (Sherwood et al., 2003) and tabletability (Chen & Chou, 1998) of amorphous APIs and at that time hot-melt technologies were very innovative techniques (Ghebre-Sellassie et al., 2004). Later, special preparation methods for came to the forefront of amorphous patents, e.g. amorphization with electrospininng (Ignatious et al., 2006), the preparation of coated implantable medical devices by a solvent-free method (Maryanoff et al., 2008) or the amorphization of pure API (esomeprazole) (Reddy et al., 2009) and stabilization (Yu et al., 2008). Nowadays, glassy drugs often feature in patents because of the better dissolution rate (Marom & Rubnov, 2012), or in patents relating to crystalline formulations, to solve problems that arise during preparation methods.

3.2 Amorphization processes in pharmaceutical technology

In pharmaceutical formulations, it is necessary to differentiate two possibilities: when an amorphous form of the API is produced alone, without the use of any auxiliary agents (Fix & Steffens, 2004; Ambike et al., 2005), when a composition involves both the amorphized API and an auxiliary material(s) as crystallization inhibitor(s). A product made in this way can be a solid dispersion (Rupprecht & Kindl, 1974) or a solid solution or some other multiple system (Bettinetti et al., 2006).

The technologies applied to make an amorphous form in pharmaceutical technology, are a solvent method (Panchagnula & Bhardwaj, 2008), hot-melt technology (Kinoshita et al., 2003) and milling processes (Delogu et al., 2004). **Figure 1** summarizes the possibilities.

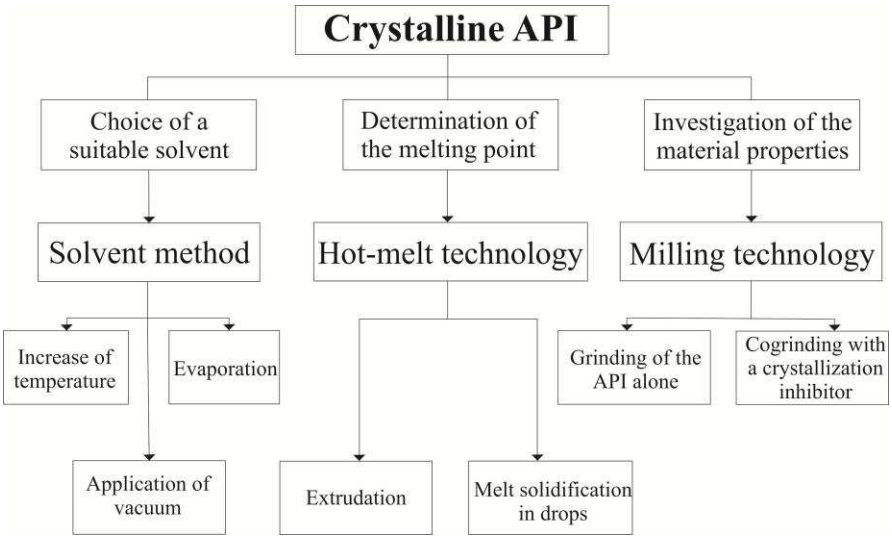

FIGURE 1: Methods for the preparation of amorphous materials

3.2.1 Solvent method

Solvent methods are among of the simplest processes utilized for amorphization in industry. It is important that the agent must dissolve without leaving a residue. If crystals remain in the system, these could start nucleation by acting as seeding crystals in the phase of supersaturation during removal of the solvent. If a solvent is applied in a pharmaceutical technological method, the danger class of this solvent must be taken into consideration according to the ICH Q3C guideline (ICH Q3C, 1998). The objective of this guideline is to recommend acceptable amounts for residual solvents in pharmaceuticals for the safety of the patient.

In the next step, the solvent has to be removed from the system. The removal must be as fast as possible, because in this way the building of the crystal lattice can be inhibited. The solvent can be removed by heating, by reducing the pressure or by spray-drying. The technological processes are outline in **Figure 2**. The literature presents many examples of amorphization with solvent technology. **Table I** lists some APIs amorphized by a solvent method.

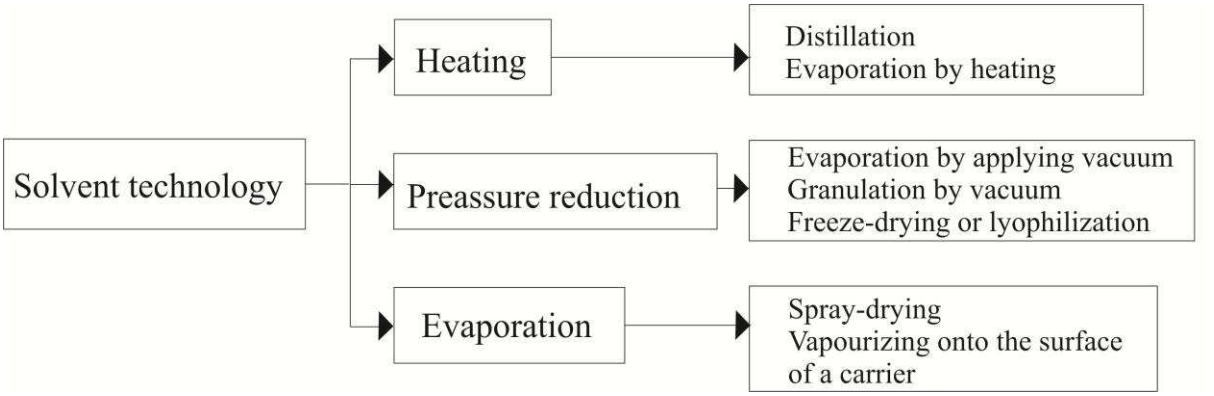

FIGURE 2: Methods of solvent technology

Table I: Amorphization of APIs with solvent technology

Amorphized API	Applied technology	Applied additive(s)	Literature reference
Atrovastatin	Spray-drying SAS process	-	Kim et al. , 2008
Carbamazepine	Solvent evaporation	PVP	Sethia & Squillante, 2004
Fenofibrate	Spray-drying	Lactose and SLS	Vogt et al., 2008
Indomethacin	Spray-drying	Nonporous or porous silica	Takeuchi et al., 2005
Ketoprofen	Solvent evaporation under reduced pressure at 40 °C	PVP	Martino et al., 2004
Loperamide	Spray-drying	PVP	Weuts et al., 2004
Paracetamol	SAS process	-	Rossmann et al., 2012
Piroxicam	RESS	Benzoic acid	Vemavarapu et al., 2009
Piroxicam	Solvent removal by reduced pressure at 40 °C	PVP	Tantishaiyakul et al., 1999
Tacrolimus	Solvent evaporation	PEG 6000 or PVP or HPMC	Yamashita et al., 2003
Tolbutamide	Spray-drying	nonporous or porous silica	Takeuchi et al., 2004

PVP: poly(vinyl pyrrolidone), RESS: rapid expansion of supercritical solution, SAS: supercritical antisolvent, PEG: poly(ethylene glycol), HPMC: hydroxypropylmethylcellulose, SLS: sodium laurylsulfate

3.2.2 Hot-melt technology

Hot-melt technologies are currently popular in the pharmaceutical industry because these methods are free from solvents. This is also important environmentally and additionally residual solvent will not be present in the product. For the applicability of this route, first of

all, the melting point (T_m) must be determined and also the temperature at which the API decomposes. We have to differentiate two processes: when the API is melted without auxiliary agent and then cooled on a cold surface (quench-cooling technology), and when the active ingredient is dissolved in a molten auxiliary agent and then the product is then solidified immediately by cooling. In this method, surfactants too can be applied. **Figure 3** illustrates the processes for hot-melt technologies. In **Table II** details some APIs which are amorphized by hot-melt technology.

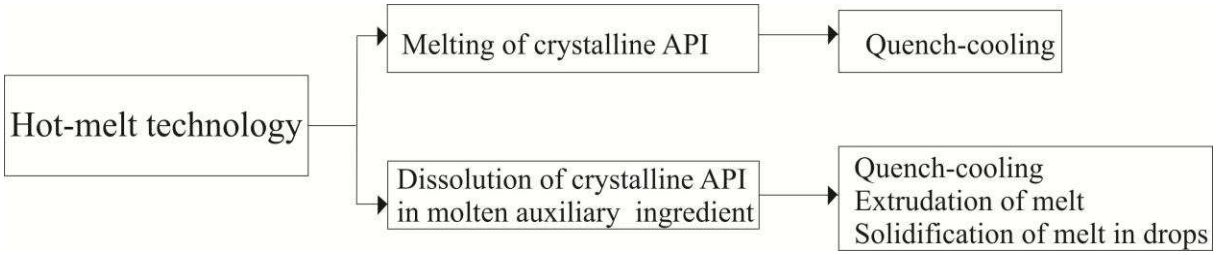

FIGURE 3: Hot-melt technology

Table II: Amorphization of APIs with hot melt technology

Amorphized API	Applied technology	Applied additive(s)	Literature reference
Carvedinol	Quenching of melt	-	Pokharkar et al., 2006
Guaifenesin	Hot-melt extrusion	Acryl-Eze or Eudragit L100-55	Bruce et al., 2007
Halofantrine	Fusion method	Gelucire 44/14 and vitamin E TPGS and PEG 6000	Khoo et al., 2000
Indomethacin	Hot-melt extrusion	PEG 8000 or PEG 10000 or PVP	Forster et al., 2001
Itraconazole	Quench-cooling	-	Weuts et al., 2003
Ketconazole	Quench-cooling	-	Weuts et al., 2003
Lacidipine	Hot-melt extrusion	Citric acid or glucose or anhydrous lactose or mannitol or PVP	Forster et al., 2001
Metoprolol	Hot-melt extrusion	PVA and PVP	Saerens et al., 2012
Miconazole	Quench-cooling	-	Weuts et al., 2003
Simvastatin	Quenching of melt	-	Ambike et al., 2005
TAS-301	Melt-adsorbed technique	Porous calcium silicate	Kinosita et al., 2003

PVA: poly(vinyl acetate), PVP: poly(vinyl pyrrolidone), PEG: poly(ethylene glycol), TAS-301: 3-bis(4-methoxyphenyl)methylene-2-indolinone

3.2.3 Milling technology

Grinding or milling is commonly used in pharmaceutical technology. With this procedure, amorphous forms can also be prepared occasionally. The efficiency of grinding depends on the structural properties of the drug materials. It is influenced by the hardness of the API, which can be characterized on the Mohs scale ranging from 1 (very soft) to 10 (very hard). This scale is used to characterize both minerals and pharmaceutical compounds. Rigid or hard crystals, or crystals containing many lattice defects, can be ground more effectively. The technical equipment applied can be ball mills or vibration mills or other grinders. Here again there are possibilities: APIs can be ground alone or can be co-ground with auxiliary agents, which can protect the amorphized materials from recrystallization. **Figure 4** summarizes the milling technologies. **Table III** contains some literature examples of amorphization by grinding technology.

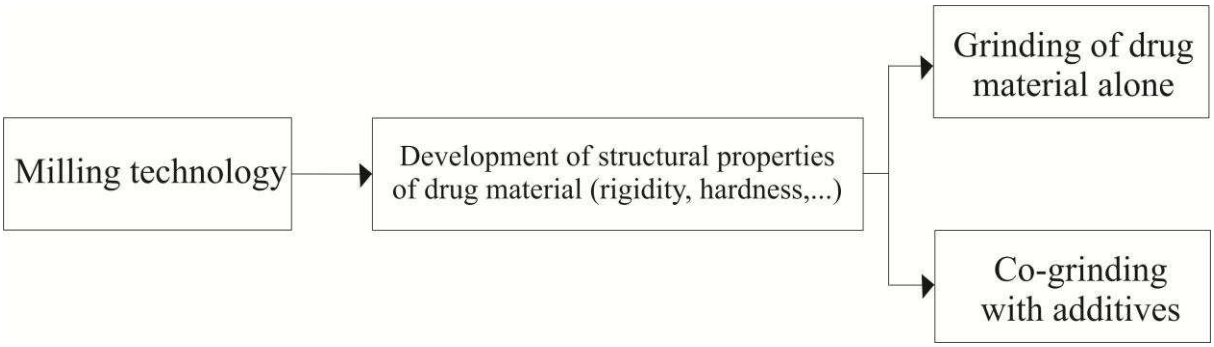

FIGURE 4: Milling technology

Table III: Amorphization of APIs by milling technology

Amorphized API	Applied technology	Applied additive(s)	Literature reference
Ibuprofen	Co-grinding in a ball mill	Kaolin	Mallick et al., 2008
Indomethacin	Co-grinding in a vibration mill	Non-porous silica	Watanabe et al., 2001
Indomethacin	Co-grinding in a vibration mill	PVP	Watanabe et al., 2003
Indomethacin	Co-grinding in a vibration mill	the mixture of nonporous silica and talc	Watanabe et al., 2002
Indomethacin	Cryogrinding	-	Hédoux et al., 2009
Meloxicam	Co-grinding in a planetary monomill	PVP and PEG	Kürti et al., 2011

PVP: poly(vinyl pyrrolidone), PEG: poly(ethylene glycol)

3.3 Classification of materials from the aspect of amorphization

The amorphous form has a higher free energy, enthalpy and entropy and a greater volume corresponding crystalline phase (**Figure 5**). On increase of temperature, the free energy decreases and the enthalpy, entropy and volume increase. If the entropy changes of the amorphous form are extrapolated to the curve of the entropy changes of the crystalline form, we obtain the Kauzmann temperature (T_K). At this point, the crystalline and the amorphous forms have the same entropy volume. When a material is stored below T_K, the amorphous form is more stable than the corresponding crystalline form because its entropy is lower than that of the crystalline form. In the literature, this rule is referred to as the Kauzmann paradox (Kauzmann, 1948).

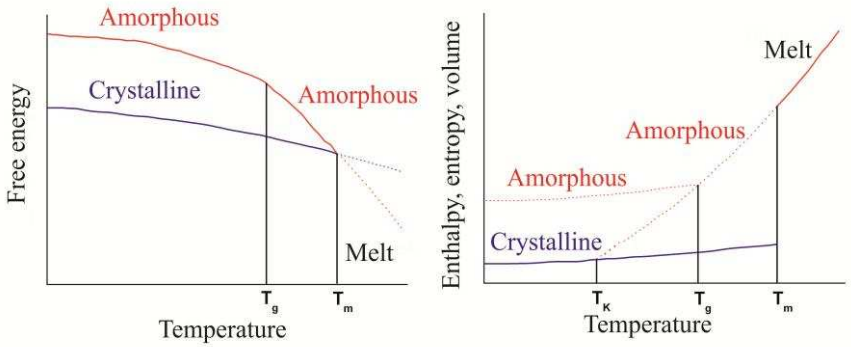

FIGURE 5: Temperature parameters of crystalline and amorphous forms. T_g: glass transition temperature; T_m: melting point; T_K: Kauzmann temperature (Kauzmann, 1948).

Crystalline systems can be characterized by their melting points (T_m), but amorphous materials do not possess this characteristic temperature parameter. They can rather be defined by the glass transition temperature (T_g), below which amorphous materials are brittle and above which they are in a liquid or rubbery state. T_g lies in the interval from approximately 2/3 to 4/5 of the T_m (in Kelvin) (Yu, 2001). For investigations of the amorphous form and T_g, differential scanning calorimetry (DSC) is a suitable experimental method (Giron, 2002, Gombás et al., 2002).

Crystalline agents can be divided into poor (or fragile) glass-formers and good (or strong) glass-formers.

For **poor or fragile glass-formers**, $\frac{Tg}{Tm} > 0.7$;

for **good or strong glass-formers**, $\frac{Tg}{Tm} < 0.7$ (Kerč & Srčič, 1995).

(In other publications, the limit is given as 1.5 for T_m/T_g: when $T_m/T_g>1.5$, the material is a good glass-former (Craig et al., 1999, Hancock & Zografi, 1997.) This allows a prediction of the amorphization properties of APIs. Good glass-formers exhibit minimal molecular mobility changes at T_g, and hence the shift in heat capacity tends to be small. However, this complicates the determination of this characteristic parameter of the glassy form.

In an amorphous system containing several amorphous components, we can define T_g(mix) through the Gordon-Taylor equation.

$$T_g\,(\text{mix}) = \frac{\left[\left(\omega_1 \cdot T_{g1}\right) + \left(K \cdot \omega_2 \cdot T_{g2}\right)\right]}{\left[\omega_1 + \left(K \cdot \omega_2\right)\right]},$$

$$\text{where } K = \frac{\rho_1 \cdot T_{g1}}{\rho_2 \cdot T_{g2}},$$

$\omega =$ mass of components,

$T_g =$ glass transition temperatures of components,

$\rho =$ amorphous densities of components (Gordon & Taylor, 1952).

The T_g - 50 K rule helps us suitable storage temperature for amorphous APIs. At this temperature, glassy APIs have “zero” structural mobility, and storage at this temperature can therefore stabilize the glassy state.

3.4 Investigations of amorphous materials

A wide array of methods are available for the solid phase analysis of pharmaceutical compounds. Techniques commonly used to study solid-state properties are listed in **Table IV** (Giron et al., 2004). The method of choice for a specific case depends on the key parameters needed to be determined and how deeply they have to be investigated. Usually, it is advisable to use two or more complementary methods to obtain a reliable knowledge of the forms (Aaltonen et al., 2009).

As concerns the amorphization of a crystalline substance an important factor is the ratio crystalline/amorphous content (Giron et al., 2007). The ICH Q2 guidelines contain the rules for the quantification and validation of methods. The industrial gold-standard for structural determination is X-ray powder diffraction (XRPD) (Gaisford, 2012). This method is often applied to quantify the amorphous form content. DSC is also an accepted method, but the information provided by DSC measurements is usually semiquantitative.

Table IV: Methods of investigation of solid states

Method	Data measured	Property measured/used	Example
X-ray diffraction (single crystal and XRPD)	Diffractogram	Crystallographic properties, quantification of amorphous/crystalline ratio	Li, 2000; Chen et al., 2001
Infrared (IR) spectroscopy	IR spectrum	Chemical information	Ambike et al., 2004
Raman spectroscopy	Raman spectrum	Chemical information (complementary to IR), quantification of amorphous/crystalline ratio	Taylor & Zografi, 1998; Widjaja et al., 2011
Tetrahertz pulsed spectroscopy (TPS)	Tetrahertz pulsed spectrum	Chemical information	Strachan et al., 2004
Near-infrared spectroscopy (NIR)	Near-infrared spectrum	Chemical information, quantification of amorphous/crystalline ratio	Gombás et al., 2003
Solid-state nuclear magnetic resonance (NMR)	Magnetic resonance	Chemical information, quantification of amorphous/crystalline ratio	Lefort et al., 2004
Differential scanning calorimetry (DSC)	Heat flow vs. temperature	Thermal events, quantification of amorphous/crystalline ratio	Gombás et al., 2002; Lefort et al., 2004
Thermogravimetry (TG)	Change in mass vs. temperature	Solvate/hydrate studies	Forster et al., 2001
Microscopy, polarized light microscopy (PLM), scanning electron microscopy (SEM)	Microscopy under the influence of light or electron radiation	Morphology, surface examinations, dehydration, polymorphism	Lechuga-Ballesteros et al., 2003
Moisture sorption/desorption isotherms	Change in mass vs. variable RH%	Hygroscopicity behaviour (hydrate formation, dehydration, amorphous crystallization	Bronlund & Paterson, 2004
Solubility/dissolution	Amount dissolved in different solvents or temperatures vs. time	Solubility/dissolution rate measurement	Murdande et al., 2010
Dynamic vapour sorption (DVS)	Moisture sorption isotherm	Quantification of amorphous/crystalline ratio	Vollenbroek et al., 2010
Microcalorimetry	Heat flow vs. time	Quantification of amorphous/crystalline ratio	Ahmed et al., 1996, Dilworth et al., 2004; Gaisford et al., 2012
Solution calorimetry	Heat flow during dissolution	Quantification of amorphous/crystalline ratio	Royall & Gaisford, 2005

4 MATERIALS AND METHODS

4.1 Materials

4.1.1 Clopidogrel hydrogensulfate (CLP)

In this study, the crystalline API which was subjected to amorphization was CLP (clopidogrel bisulfate), a potent oral antiplatelet agent often used in the treatment of coronary artery disease, peripheral vascular disease and cerebrovascular disease as Plavix® (an original drug product). Many generic products containing this active agent are currently on the market with in Hungary. The chemical formula of CLP is $C_{16}H_{16}ClNO_2S \cdot H_2SO_4$ and the molecular mass is 419.9. Chemically, it is classed among the thiophenes, and its systematic IUPAC name is methyl (+)-(S)-alpha-(2-chlorophenyl)-6,7-dihydrothieno[3,2-c]pyridine-5(4H)-acetate sulfate. The chemichal structure is to be seen in **Figure 6**. Six different polymorphic forms (**Table V**) and an amorphous form of the drug have been identified, but only forms I and II are used in pharmaceutical formulations (Bousquet et al., 2003; Uvarov & Popov, 2008). The polymorphic and amorphous forms of this drug are dealt with in a number of patents and articles.

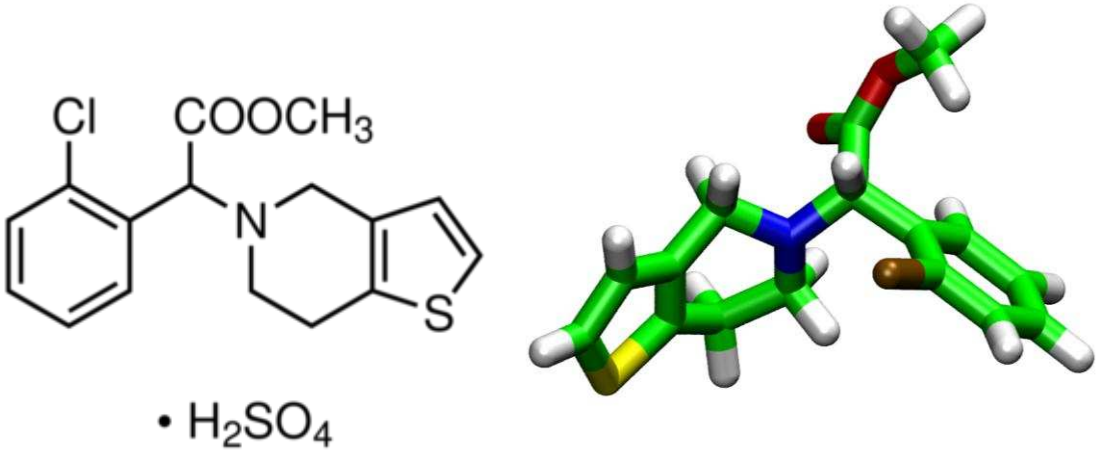

FIGURE 6: The chemical structure of CLP

Table V: Polymorphic and amorphous forms of CLP

Polymorphic form	Melting point (T_m)	Reference
I	184±3 ºC	Lohray et al., 2004
II	176±3 ºC	Lohray et al., 2004
III	105 ºC	Lifshitz et al., 2004
IV	160-170 ºC	Lifshitz et al., 2004
V	126-132 ºC	Lifshitz et al., 2004
VI	136 ºC	Lifshitz et al., 2003
Amorphous	-	Parthasaradhi et al., 2006

CLP polymorphic form II (EGIS, Budapest, Hungary) was used as the crystalline API.

4.1.2 Solvents and additives

Table VI details the solvents and additives applied and their producers.

Table VI: Applied solvents and additives

Type of additives	Materials	Producer
Solvents	Ethanol 96% v/v	Merck, Hungary
	Methanol	
	Acetone	Reanal, Hungary
Crystallization inhibitors	Aerosil 200 (colloidal SiO_2)	Nippon Aerosil Co., Japan
	Syloid 72 FP (porous SiO_2)	Grace, Hungary
	Kaolin	Merck, Hungary
	Mannitol	
	Microcrystalline cellulose (MCC) (Avicel PH 101)	FMC Corporation, Europe
	Poly(vinyl pyrrolidone) (PVP K25) (PVP, Kollidon® 25	BASF, Germany
	Cross-linked PVP (Crospovidone, Kollidon® CL-M, PVP K CL-M)	
	Methylcellulose	(Ph. Eur.)
Auxiliary agents of tablet making	Microcrystalline cellulose (MCC) (Avicel PH 101), as filler	FMC Corporation, Europe
	Cross-linked PVP (PVP Polypl. XL 10) (Polyplasdone® XL 10, N-vinyl-2-pyrrolidone polymer), as disintegrant	I.S.P. Technologies Inc., Germany
	Magnesium stearate, as lubricant	Hungaropharma, Hungary

4.2 Methods of preparation

4.2.1 Preparation of pure amorphous CLP

Amorphous samples were made with the use of ethanol 96% v/v or methanol. 1.00 g CLP was dissolved in 10.00 g ethanol or 4.00 g methanol with the aid of a magnetic stirrer (Velp® Scientifica, Europe) for 5 minutes at room temperature. The solvent was evaporated by two methods: with blown room temperature air or under vacuum (Binder, Germany). 1.00 g CLP was treated with 20.00 g acetone with magnetic mixing for 15 minutes at room temperature and the solvent was then evaporated off in vacuum (Binder, Germany). After drying, samples were pulverized in a porcelain mortar with a pestle. In the following steps, we used the sample which was prepared with ethanol and dried with room temperature air as amorphous reference sample. Ethanol was the most suitable solvent for the amorphization of CLP (see section 5.1).

4.2.2 Selection of a crystallization inhibitor

Different masses of CLP were dissolved in different amounts of ethanol 96% v/v. The resulting solutions were mixed with different crystallization inhibitors in a porcelain mortar, leading to the formation of a solution or a suspension or a gel. The ratio CLP:crystallization inhibitor was 7:3. The mixtures were then dried with room-temperature air (25 °C, 46% relative humidity (RH)). After the most suitable inhibitor had been chosen, it was mixed with CLP in ratios of 1:9; 3:7; 1:1; 7:3 and 9:1 with the aim of finding the best active API:auxiliary agent ratio.

4.2.3 Amorphization in scaling-up processes

Sample 1: 28.0 g of CLP was dissolved in 160.0 g of ethanol 96% v/v with the use of a magnetic mixer for 2 min. 12.0 g of Aerosil 200 and 40.0 g of MCC were mixed with a Turbula mixer (speed: 50 rpm, duration of mixing: 5 min). The solution of CLP was then vaporized onto the surface of the Aerosil 200-MCC mixture bed in a pan (Dragex-1, Jørgen).

Sample 2: 28.0 g of CLP was dissolved in 160.0 g of ethanol 96% v/v with the use of a magnetic mixer for 2 min. 12.0 g of Aerosil 200 was added to the solution of CLP and underwent solvation in 2 min; a gel was made by mixing. This mixture was vapourized onto the surface of 40.0 g of a MCC bed in the same pan.

The parameters (in both cases): pan (Dragex-1 stainless steel equipment, furnished with an exhauster system for removal of the solvent under processing), rotation speed: 25

rpm; pressure of spraying air: 0.1 bar; type of vapourizer: Walther, 1 mm nozzle diameter; drying air temperature: 25 ºC; RH of drying air: 46%; transportation of liquid: Peripump; speed of transportation: at the beginning of the measurement 5 ml/min; at the end of the measurement: 1 ml/min (this depends on the rate of drying). This step involves a 28-fold scaling-up. During the process, the loss of powder was very variable, depending on the situation of the Aerosil 200 (in a powder bed or in alcoholic solution).

4.2.4 Tablet-making

A larger amount of stabilized product was prepared with the production method employed for Sample 2. This product was the internal phase of the tablets. The mass of a tablet was 400 mg, containing 100 mg of CLP. The composition for 1000 tablets is given in **Table VII**. The internal and external phases were mixed with a Turbula mixer (speed: 50 rpm, duration of mixing: 5 min). Tablets were made with a Korsch EKO eccentric tablet machine (Emil Korsch Maschinenfabrik, Berlin, Germany) (35 tablets/min, punch diameter 12 mm, compression force: 9±1 kN).

TABLE VII: Composition for 1000 tablets

	Substances	**Mass**
Internal phase	CLP	100.00 g
	Aerosil 200	42.86 g
	MCC	243.14 g
External phase	Polyplasdone XL 10	12.00 g
	Magnesium stearate	2.00 g

4.3 Methods of investigations

4.3.1 Differential scanning calorimetry (DSC)

For characterization of the amorphous form, DSC was used (a Mettler-Toledo DSC 821 instrument). Approximately 4.80-5.20 mg of sample was placed into an aluminium pan, which was then sealed and scanned from 25 ºC to 200 ºC at 5 ºC/min under an argon gas flow at 100-150 ml/min. The instrument was calibrated with the use of indium.

4.3.2 X-ray powder diffraction (XRPD)

XRPD was performed with an X-ray Diffractometer Miniflex II (Rigaku, Tokyo, Japan), where the tube anode was copper with $K\alpha$=1.5405 Å. The pattern was collected at a tube voltage of 30 kV and a tube current of 15 mA in step scan mode ($4°min^{-1}$). The instrument was calibrated with silicon.

4.3.3 FT-IR analysis

To demonstrate, that no degradation occurred during preparation, and for the chemical stability testing of samples, we used an FT-IR apparatus, Avatar 330 FT-IR spectrometer (Thermo Nicolet, USA). The sample, with a CLP content of 0.5 mg, was mixed with 150 mg of dry KBr in an agate mortar, and the mixture was then compressed into a disc at 10 t. Each disc was scanned 64 times at a resolution of 2 cm^{-1} over the wavenumber region 4000–400 cm^{-1}.

The presence of H-bonding in the samples was confirmed by FT-IR analysis in the solid and in the liquid phase. Dichloromethane was applied as solvent for the preparation of solutions. In the liquid phase, the concentrations were 0.1000 g, 0.0500 g, 0.0250 g or 0.0125 g in 10 cm^3. IR spectra were recorded 4000 and 400 cm^{-1} on a FT-IR spectrometer (Bio-Rad Digilab Division FTS-65A/869, USA) between. The spectrometer was equipped with a DTGS detector for the measurements on solid samples. Solutions were investigated in a KBr liquid cell of 0.1 mm in thickness. The spectral resolution was 4 cm^{-1} and 128 scans were averaged.

4.3.4 Investigation of tablet parameters

Five parameters of the tablets were investigated: mass, diameter, height (measured with a screw micrometer; Mitutoyo Corporation, Tokyo, Japan), hardness against pressure (Heberlein apparatus, Le Locle, Switzerland) and the time of disintegration (Erweka ZT71, GmbH, Germany). Investigations were made with fresh and with stored tablets.

4.3.5 Investigation of stability of products

As recommended by international guidelines (ICH Q1A), we stored samples under two different conditions. Long-term testing was performed at 25±2 ºC with 60±5% RH, and accelerated testing at 40±2 ºC with 75±5% RH. Under both conditions, samples were stored in open and in closed containers; the duration of storage was 4 weeks.

5 RESULTS

5.1 Confirmation of amorphous form

Prepared samples were measured primarily by DSC. With this method, characterization of the amorphous form is possible quickly. The starting material and 5 prepared samples were tested at first by DSC. These curves are presented in **Figure 7**. The crystalline CLP melted 177.4 ºC (450.6 K). The normalized heat capacity change was 83.9 Jg^{-1}. The sample which was treated with acetone remained in the crystalline phase. The melting point of this material was 177.8 ºC (450.9 K) and the normalized heat capacity change was 82.6 Jg^{-1}. The samples prepared in ethanol or methanol was transformed to the amorphous form both on drying through blowing with room-temperature air and under vacuum. The characteristic melting point disappeared completely from the DSC curves, which were virtually straight lines without any enthalpy changes; no T_g could be detected.

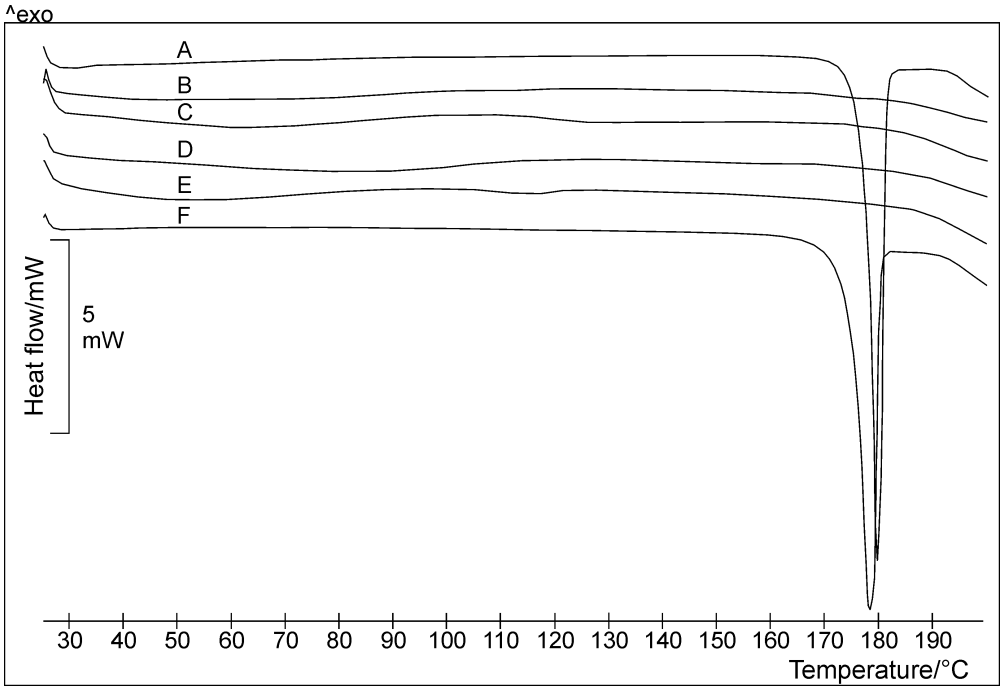

FIGURE 7: CLP samples measured by DSC. **A**: Crystalline form, **B**: sample prepared with ethanol, dried by blowing of room-temperature air, **C**: sample prepared with ethanol, dried under vacuum, **D**: sample prepared with methanol, dried by blowing of room-temperature air, **E**: sample prepared with methanol, dried under vacuum, **F**: sample treated with acetone, dried under vacuum.

Samples were also tested by XRPD measurement. Diffractograms are to be seen in **Figure 8**; for clarity, the diffractograms are displaced along the y axis. This investigation supported the DSC results throughout. **Figure 8** shows that the products prepared with ethanol or methanol, independently of the drying procedure, were converted to the amorphous form, because the peaks disappeared from the diffractograms, and the spectra became smooth. The sample treated with acetone remained in the crystalline phase, and the diffractogram of this preparation was the same as that of the crystalline starting material.

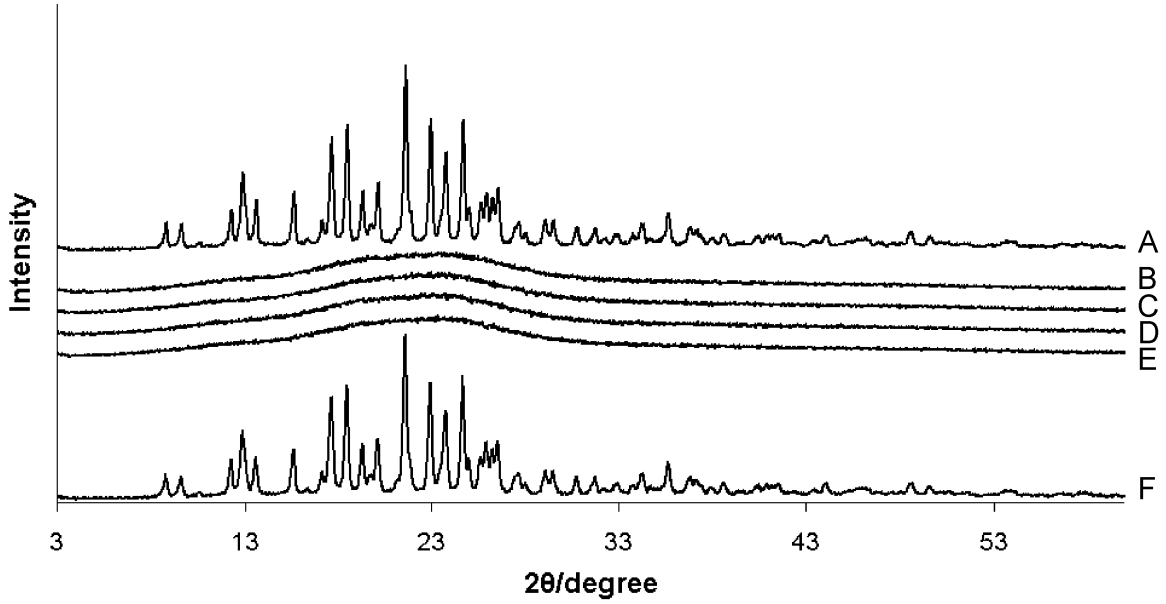

FIGURE 8: CLP samples measured by XRPD. **A**: Crystalline form, **B**: sample prepared with ethanol, dried with room-temperature air, **C**: sample prepared with ethanol, dried under vacuum, **D**: sample prepared with methanol, dried with room-temperature air, **E**: sample prepared with methanol, dried under vacuum, **F**: sample treated with acetone, dried under vacuum.

To confirm that no degradation had occurred in the prepared samples, FT-IR analysis was performed (**Figure 9**). For clarity, these spectra too have been displaced along the y axis. Each peak was present in each spectrum, reflecting the presence of the same chemical bonds, and no degradation could be detected in the course of these measurements. The amorphous and crystalline materials furnished the same spectra. Thus, as we had anticipated, with this method we could not differentiate the crystalline and the amorphous forms, but we confirmed that no degradation had taken place in the samples.

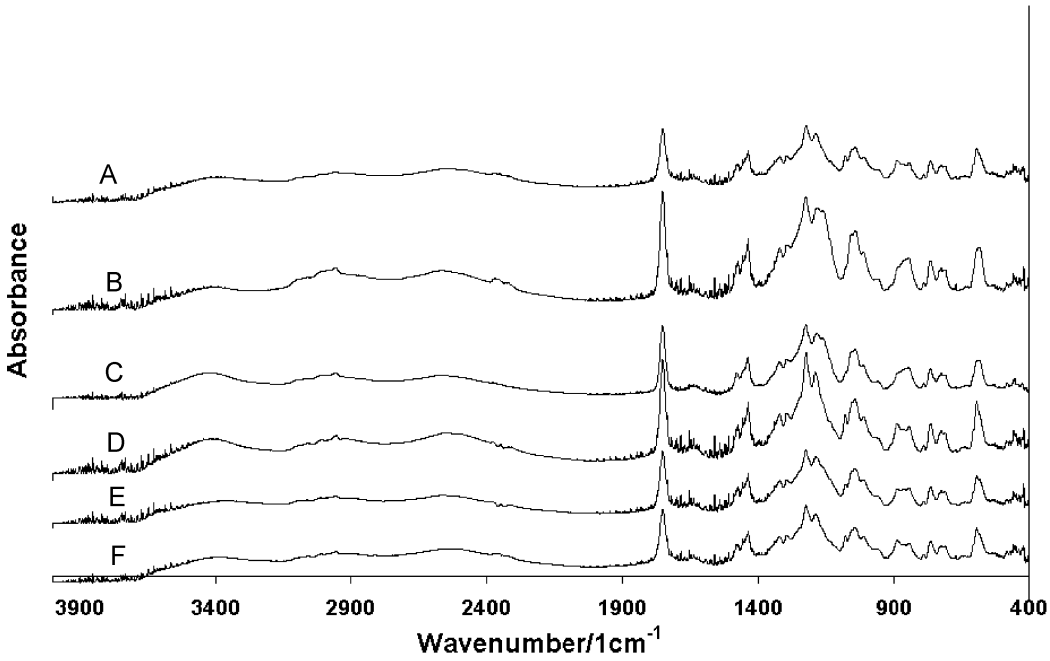

FIGURE 9: CLP samples measured by FT-IR. **A**: Crystalline form (II), **B**: sample prepared with ethanol, dried with room-temperature air, **C**: sample prepared with ethanol, dried under vacuum, **D**: sample prepared with methanol, dried with room-temperature air, **E**: sample prepared with methanol, dried under vacuum, **F**: sample treated with acetone, dried under vacuum.

The DSC, XRPD and FT-IR results suggested that the samples prepared with ethanol or methanol were transformed to the amorphous form independently of the drying procedure, but the sample treated with acetone remained in the crystalline form. Acetone is not a suitable solvent for the amorphization of CLP, but ethanol and methanol have the same amorphizing property in the case of this API. As mentioned in the literature background, if we apply a solvent in a pharmaceutical technological method, we must take into account the danger class of this solvent according to the ICH Q3C guideline (1998). The objective of this guideline is to recommend acceptable amounts for residual solvents in pharmaceuticals for the safety of the patient. ICH Q3C classifies ethanol into the less dangerous Class 3, while methanol is classified into the more dangerous Class 2. Accordingly, we recomended the application of ethanol for the amorphization of CLP.

5.2 Measurement of T_g

T_g is one of the most important parameters for amorphous materials. The expected temperature interval in which T_g can lie is approximately 2/3 to 4/5 of T_m (in Kelvin) (Yu, 2001). The T_m of crystalline CLP is 177.4 ºC (450.6 K). Accordingly, the expected interval of glass transition is 27.2-87.3 ºC (300.4-360.4 K).

A DSC curve reveals all structural changes accompanied by enthalpy changes. In the curve, T_g is usually indicated by a step, a dislocation from the baseline. For CLP, however, T_g could not be detected during the first heating run T_g, but during the second heating T_g appeared in the curve (**Figure 10**). When double heating was carried out with two samples with ethanol, the endothermic step in the DSC curve was detected in the same interval. This temperature interval, 82-110 ºC (355-383 K) can be defined as the glass transition of CLP. The midpoints of these changes were 89.4 ºC (362.6 K) and 88.5 ºC (361.6 K), with a mean of 88.9 ºC (362.1 K). Thus, for the calculations, this mean value (88.9 ºC = 362.1 K) was applied as T_g. For CLP, therefore the quotient T_g/T_m is 0.80, and accordingly CLP can be classified as a good glass-former.

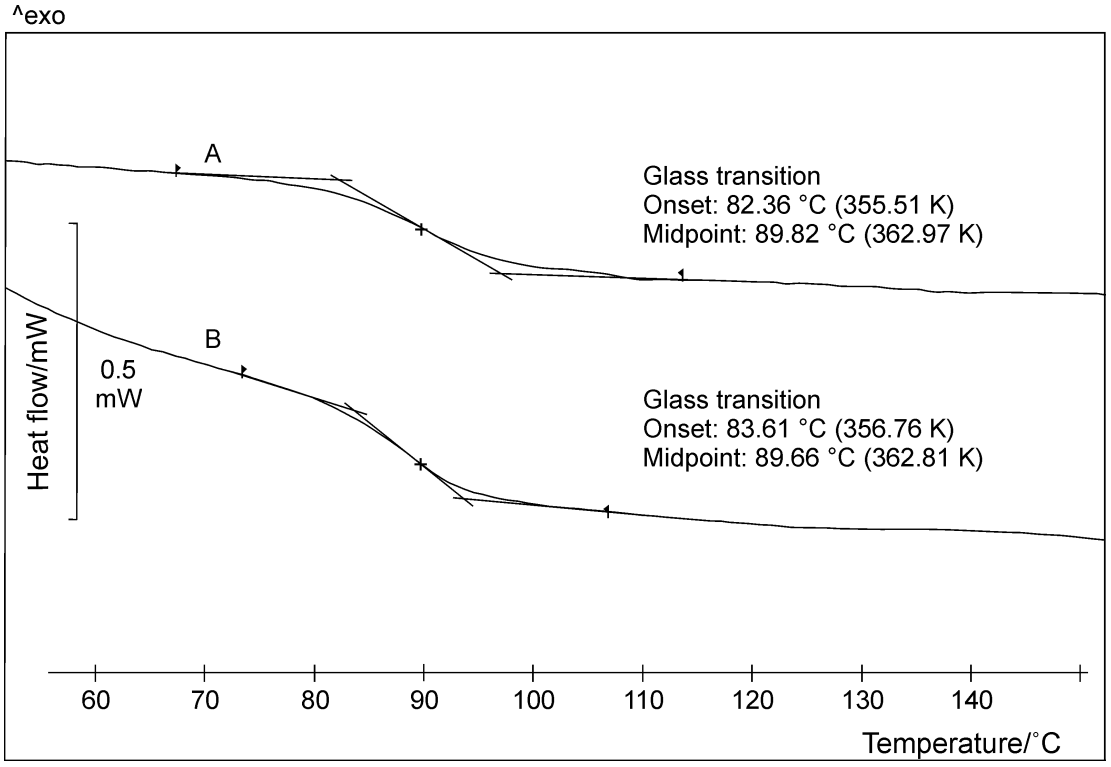

FIGURE 10: Glass transition temperature of CLP measured by DSC. **A**: Sample prepared with ethanol, dried with room-temperature air, **B**: sample prepared with ethanol, dried under vacuum.

5.3 Preliminary stability testing

In the case of amorphous materials, stability problems can occur because of the higher energy level. The possibility of recrystallization is very realistic. The amorphous CLP was subjected to preliminary stability testing. A sample prepared with ethanol was stored in a closed glass container at 23±2 ºC and 55±5 RH. It was observed that crystal growth started after 30 days (**Figure 11**). The crystallinity of the sample increased for approximately 76 days and the crystal growth then stopped. The characteristic T_m appeared in curve B at 172 ºC (445.2 K). This peak increased linearly with time, but the change stopped after 76 days. T_m was found in the interval 170-172 ºC (443.2-445.2 K). Values of ΔH are given in **Table VIII**. It was clear that recrystallization started in the amorphized sample.

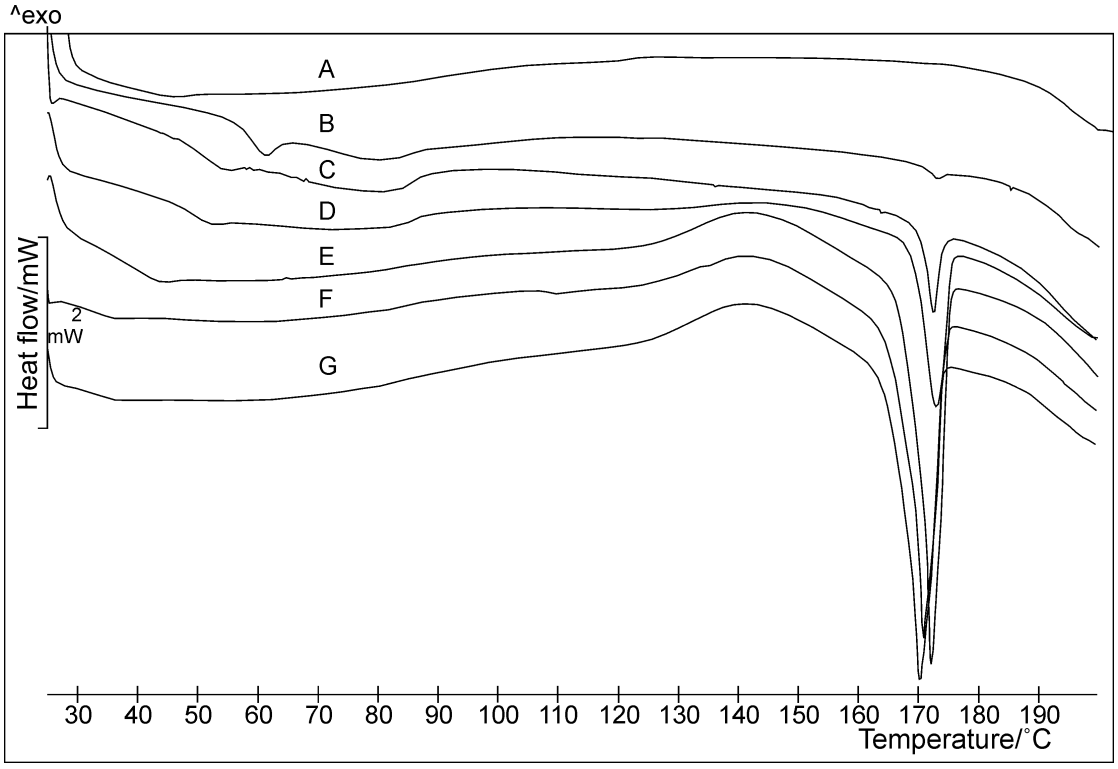

FIGURE 11: Preliminary stability testing: A: Fresh sample; B: sample stored for 30 days; C: sample stored for 34 days; D: sample stored for 45 days; E: sample stored for 76 days; F: sample stored for 96 days; G: sample stored for 109 days.

Table VIII: Preliminary stability testing: Changes in ΔH determined by DSC measurement

Duration of storage/ day	ΔH/Jg^{-1}
0	0.00
30	0.55
34	4.10
45	15.82
76	41.65
96	42.64
109	40.96

The results showed that amorphous CLP can not be used as a pure drug material in pharmaceutical formulations because of its relatively fast recrystallization.

5.4 Selection of a crystallization inhibitor

Because of the fast recrystallization, the stability of CLP must be increased through the use of a crystallization inhibitor, which is the auxiliary agent in the tablet composition. In this step, different crystallization inhibitors were tested (**Table VI**). These auxiliary agents can be classified as crystalline (e.g. mannitol), semicrystalline (e.g. MCC) or amorphous (e.g. Aerosil 200) materials. Their common property is a large specific surface and they undergo physical interactions (secondary bonding) with numerous materials. These properties can prevent the building-up of crystals and the development of the long-range order of molecules of APIs.

DSC curves of the reference CLP (crystalline and amorphous) and samples containing CLP can be seen in **Figure 12**. The thermogram of crystalline CLP exhibited a sharp endothermic peak at 177.4 ºC, corresponding to the melting point of CLP. The scan of the amorphized reference CLP (made with ethanol) did not contain any characteristic peak, of course. The other samples were made with a CLP:auxiliary agent ratio of 7:3. For the sample in which mannitol was present as crystallization inhibitor, the peak was situated at about 146 ºC, due to the dissolution of CLP in the melted mannitol (T_m for mannitol is 165 ºC) (Gombás et al., 2003). The samples with kaolin and MCC exhibited decreased CLP T_m values

(168 and 169 ºC). In this range, these agents do not have melting points. The samples containing PVP K25 or PVP K CL-M and CLP in amorphous form, underwent a colour change because of incompatibility between the components. The curve for the sample made with methylcellulose displayed a double peak effect, at 167.9 ºC and 177.6 ºC. This may be an indication of two polymorphs in this sample. It has been reported that polymorphic form IV melts at 167.9 ºC (Lifshitz et al., 2004) and form II at 177.6 ºC (Lohray et al., 2004). Accordingly, this sample contains three different forms of CLP: the amorphous form, and the crystalline forms IV and II. Aerosil 200 and Syloid 72 FP differ greatly in applicability as crystallization inhibitors, despite both of them consisting of SiO_2. With ethanol as solvent, Aerosil 200 resulted in perfectly amorphous CLP, in contrast with Syloid 72 FP, which amorphized the CLP only partially. This result can be explained as a consequence of the gelling property of Aerosil 200 in ethanol. After the evaporation of the ethanol, the large surface of SiO_2 fixes the CLP and protects it against crystal growth, because of the interaction between CLP and SiO_2. This interaction presumably involves H-bonding with the surface silanol groups on the SiO_2. Such silanol groups are not present on the surface of Syloid 72 FP, which rules out this interaction. This supposition is based on the reported verification of the presence of H-bonding between indomethacin and SiO_2 by solid-state NMR imaging (Watanabe et al., 2001). These results led us to choose Aerosil 200 as crystallization inhibitor for the scaling-up process. This auxiliary agent is a classical additive in pharmaceutical formulations. In the case of solid forms, it can be used as a glidant (Ohta et al., 2003) or a coating material (Tayel et al., 2008) in tablet making, as a surface modifier (e. g. in dry powder inhalation (DPI) formulations, due to its highly hydrophilic and adsorbing properties) (Kawashima et al., 1998), and as an auxiliary agent in the preparation of solid dispersions (Takeuchi et al., 2004). It is an amorphous agent itself, and thus the presence of API crystals in samples can be detected unambiguously, e.g. by DSC.

The following step in this work was to find the optimum CLP:Aerosil 200 ratio. Five different compositions were tested with this aim. The DSC curves are presented in **Figure 13**. Melting is not detected in curves **A**, **B**, **C**, **D** and **E**, and these samples can therefore be regarded as amorphous. Curve **F**, which relates to a CLP:Aerosil 200 ratio of 9:1, indicates T_m at 169.5 ºC, which means the recrystallization of CLP. Accordingly, this amount of crystallization inhibitor is not sufficient to maintain the active agent in amorphous form. For this reason, a CLP:SiO_2 ratio of 7:3 was chosen for tablet formulation.

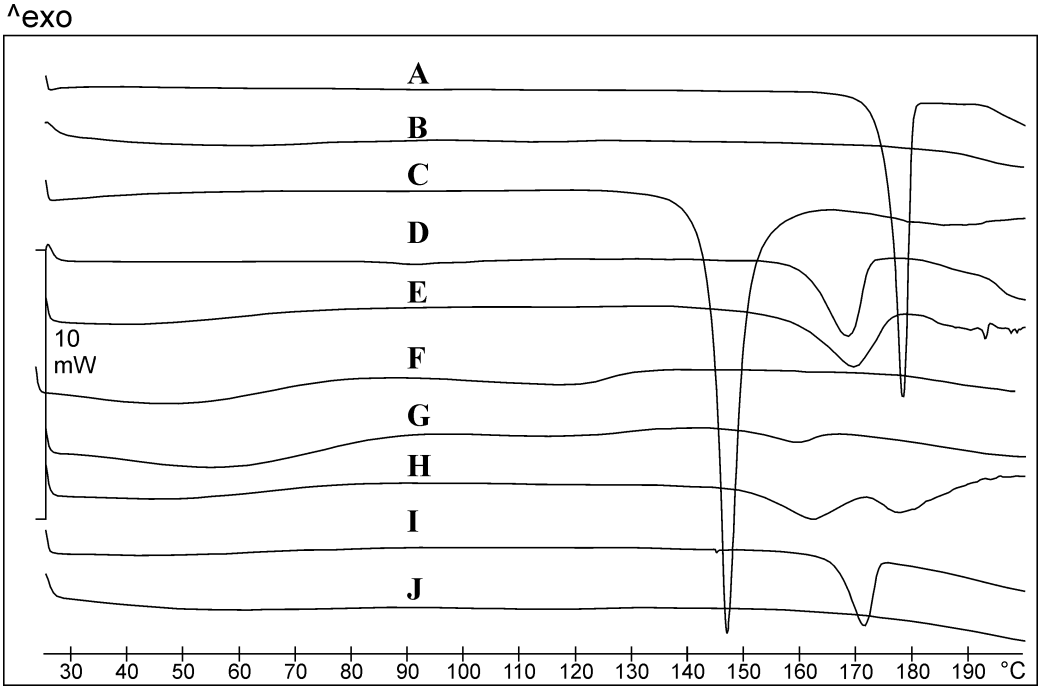

FIGURE 12: Effects of crystallization inhibitors on the crystallinity of CLP. DSC curves of reference materials and samples made with different crystallization inhibitors. **A**: Crystalline reference sample, **B**: amorphous reference sample, **C**: sample with mannitol, **D**: sample with kaolin, **E**: sample with MCC, **F**: sample with PVP K25, **G**: sample with PVP K CL-M, **H**: sample with methylcellulose, **I**: sample with Syloid 72 FP, and **J**: sample with Aerosil 200.

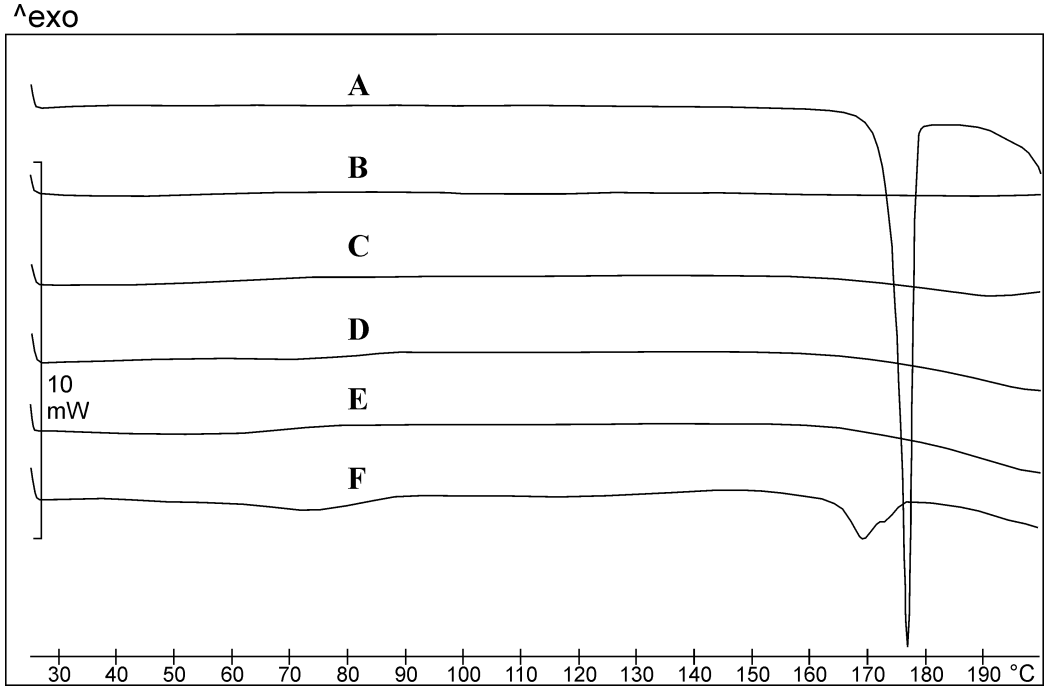

FIGURE 13: DSC curves of samples containing Aerosil 200. **A**: Reference (crystalline CLP), **B**: CLP:SiO_2=1:9, **C**: CLP:SiO_2=3:7, **D**: CLP:SiO_2=1:1, **E**: CLP:SiO_2=7:3, **F**: CLP:SiO_2=9:1.

5.5 Amorphization in scaling-up processes

The next step was to stabilize amorphous CLP on the surface of the carrier. In this system, MCC was used as the carrier, which serves as a filler/binder in tablet making. In Sample 1, an alcoholic solution of CLP was vapourized onto the surface of a mixture of MCC and Aerosil 200. With this preparation procedure, the powder underwent considerable outflow from the pan (powder effusing or dusting). The yield of the preparation was only 64.8%. For Sample 2, only MCC was added to the pan. The mixture of CLP and Aerosil 200 was dissolved in ethanol (96% v/v) and vapourized onto a MCC bed. The yield of this preparation method was 85.2%, clearly indicating that the processing of Aerosil 200 in the liquid phase is more advantageous. The DSC scans of both samples and a physical mixture (**Figure 14**) demonstrated that the CLP in both samples was in the amorphous form (in contrast with the situation for the physical mixture) because there was no sign of T_m in the curves.

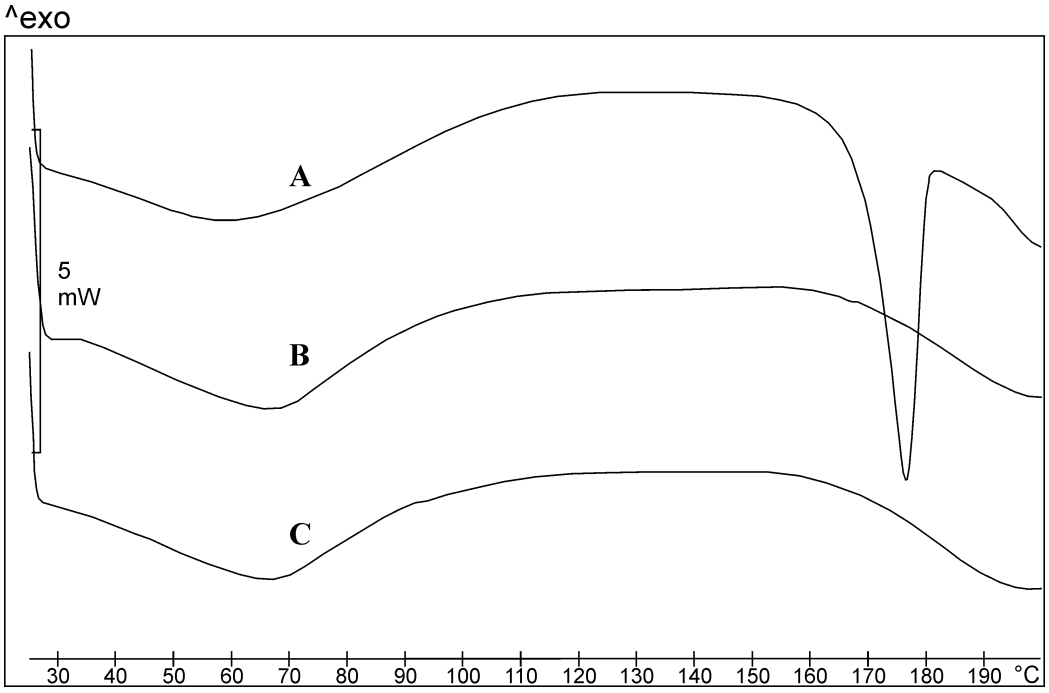

FIGURE 14: DSC curves of physical mixture and Sample 1 and Sample 2. **A**: Physical mixture, **B**: Sample 1, **C**: Sample 2

To verify H-bonding between the silanol groups of the Aerosil 200 and the CLP molecules, we compared the FT-IR spectra of Sample 2 and the corresponding physical mixture (**Figure 15**). In the interval of 900-600 cm^{-1}, spectrum **A** contains vibrations relating

to deformation of C structure. These bands are markedly decreased in spectrum **B**, indicating that the product is in the amorphous state. On the other hand, it also demonstrates the presence of chemical bonding in the product.

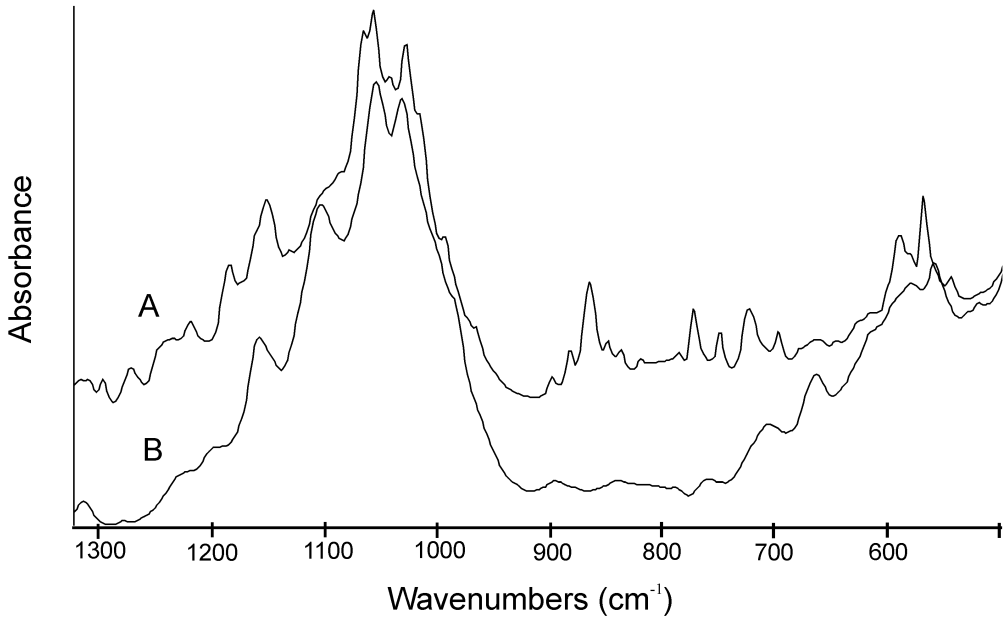

FIGURE 15: FT-IR analysis of Sample 2 and the corresponding physical mixture. **A**: Physical mixture, **B**: Sample 2.

To confirm the chemical bonding, we measured Sample 2 at different concentrations in solution (**Figure 16**). In the interval 1100-1000 cm^{-1}, spectrum **A** reveals extensive association. On dilution of the sample, this association progressively breaks down (spectra **B**, **C**, **D** and **E**). Between 1058 and 1036 cm^{-1} two bands appeared, reflecting ν_{as} C-O-C stretching (indicative of acetates). The band at 1058 cm^{-1} shifted to 1036 cm^{-1} on dilution, demonstrating the break-down of the association. These results confirm the presence of H-bonding in the solid Sample 2. The different states of the samples lead to the different positions of the bands. Spectrum **F** shows that dichloromethane does not give a signal in this interval.

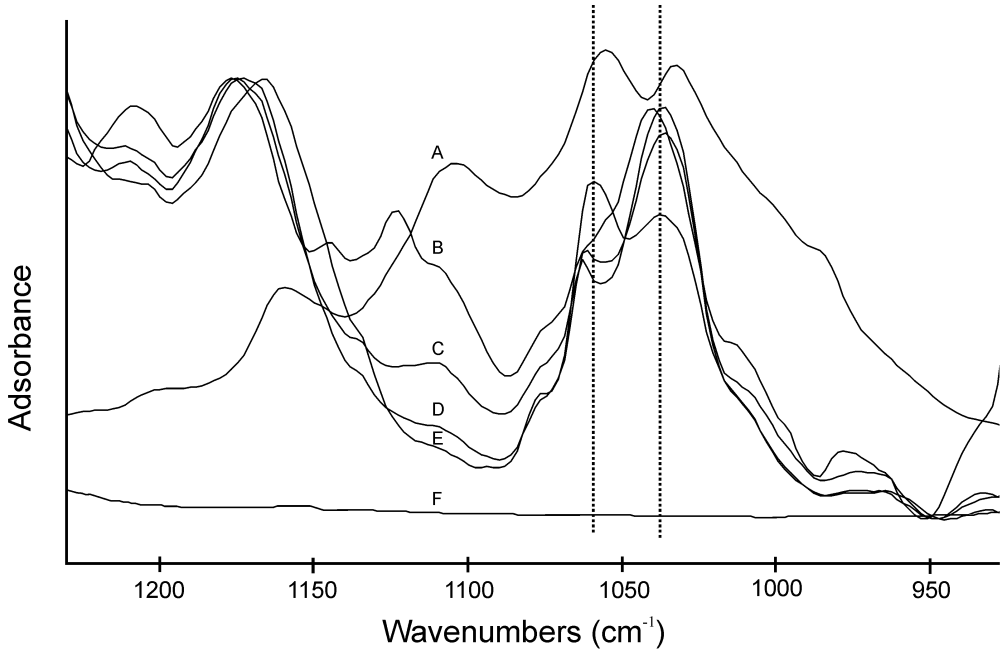

FIGURE 16: FT-IR analysis of Sample 2 at different concentrations in solutions containing dichloromethane. **A**: Solid Sample 2, **B**: 0.1000 g sample in 10 cm^3 of solution, **C**: 0.0500 g sample in 10 cm^3 of solution, **D**: 0.0250 g sample in 10 cm^3 of solution, **E**: 0.0125 g sample in 10 cm^3 of solution, **F**: dichloromethane.

In the study of the stability of amorphous CLP, Sample 1 and Sample 2 were stored for 4 weeks at 25 ºC and 60% RH. The results revealed that the stored samples remained in the amorphous phase. The findings of accelerated testing showed that, when Sample 1 was stored in either open or closed containers for 4 weeks, it included crystalline material. For Sample 2, only the sample stored in an open container included a crystalline phase.

To investigate the chemical stability of Sample 2, FT-IR analysis was used. The FT-IR spectra showed that there were no chemical changes in the samples stored either in open or in closed containers. All of the peaks were the same in the fresh and in the stored samples (**Figure 17**).

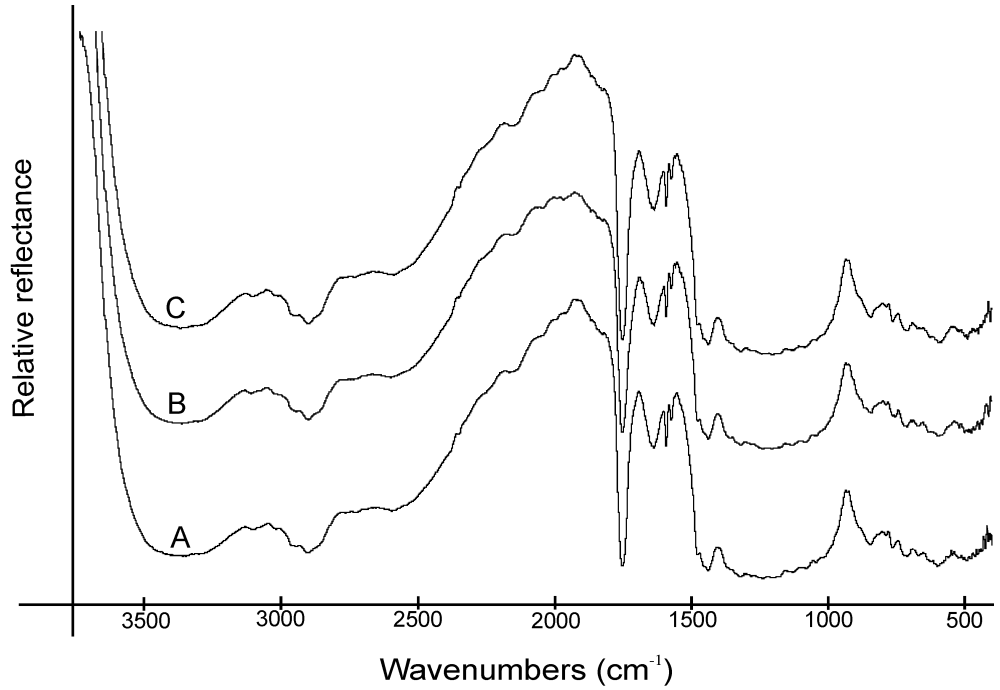

FIGURE 17: FT-IR investigation of the fresh and stored Sample 2. **A**: Fresh Sample 2, **B**: Sample 2 stored at 40 ºC, 75% RH, in an open container for 4 weeks, **C**: Sample 2 stored at 40 ºC, 75% RH in a closed container for 4 weeks.

It can be concluded that the procedure applied for the preparation of Sample 2 is better, and this product is more stable than Sample 1. In Sample 2, the gel structure of the CLP/Aerosil 200/ethanol system extends the adherence of the CLP/Aerosil 200 system on the surface of MCC. There is a H-bonding interaction between the surface silanol groups of Aerosil 200 and the hydroxy groups of MCC on the MCC surface (Jonat et al., 2006). This interaction can come into existence more easily in the liquid phase (Sample 2) than in the solid phase in the case of simple mixing (Sample 1). For these reasons, the amorphization procedure used for Sample 2 was applied in tablet making.

5.6 Preparation and investigation of tablets

The amorphization procedure applied for Sample 2 was used to make 1000 tablets (see **Table VII**).

Both fresh and stored tablets were investigated. In the thermoanalytical study, a physical mixture of the tablet components was also investigated because of the presence of the crystalline phase of CLP. The DSC curves are depicted in **Figure 18**. The slight enthalpy changes detected in curve **E** indicate that this sample may contain a little crystalline phase.

This sample was stored at 40 ºC and 75% RH in an open container for 4 weeks. Curves **A**, **B**, **C**, **D** and **F** do not reveal any crystalline phase in the system. The XRPD investigations resulted in constant data. The fingerprint of CLP did not appear in the diffractogram. A feature of importance for tablet making was that the surface area of the amorphous product decreased, which was another stabilizing step in the formulation.

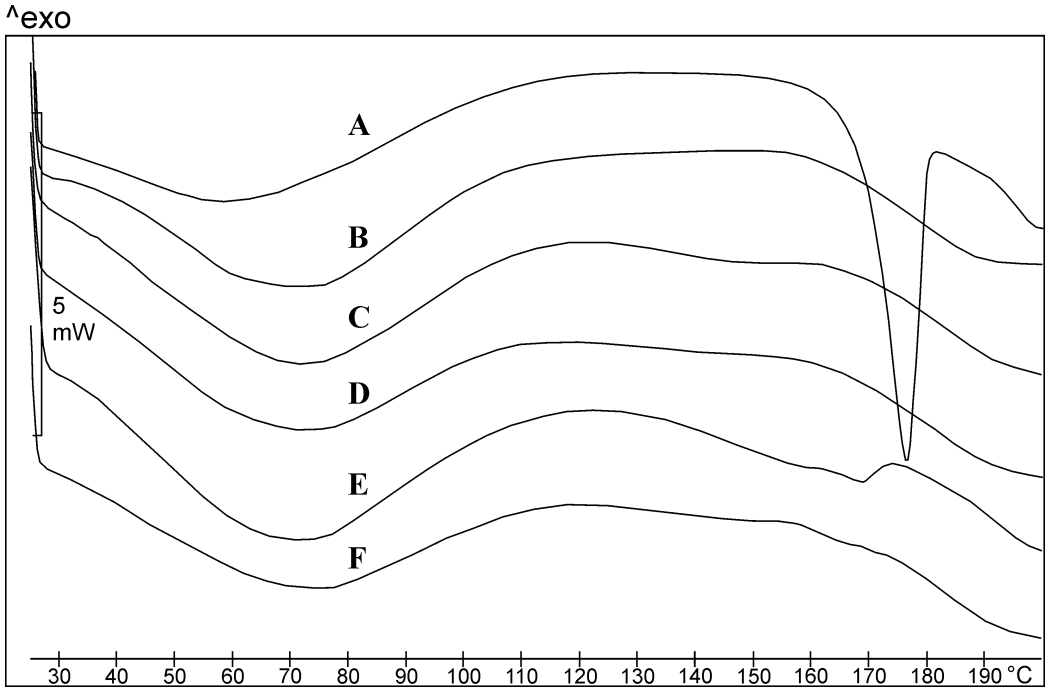

FIGURE 18: Stability of tablets. **A**: Physical mixture, **B**: fresh product, **C**: sample stored at 25 ºC, 60% RH, in an open container for 4 weeks, **D**: sample stored at 25 ºC, 60% RH in a closed container for 4 weeks, **E**: sample stored at 40 ºC, 75% RH, in an open container for 4 weeks, **F**: sample stored at 40 ºC, 75% RH, in a closed container for 4 weeks.

The physical parameters of the fresh and stored tablets are reported in **Table IX**. The change in mass of the tablets was greatest for the tablets stored in an open container for 4 weeks under accelerated conditions (40 ºC, 75% RH). As the mass of these tablets increased, the diameter and height also increased. In the other cases, the changes were negligible. The hardness against pressure of the tablets decreased in all cases, most strongly for the tablets stored in open containers. The time of disintegration also decreased in all cases, and again the most considerable changes occurred for the tablets stored in open containers. These results are

in harmony with the fact that amorphous materials are hygroscopic. In these changes, the presence of Polyplasdone XL 10, as superdisintegrant, also plays an important part.

TABLE IX: Investigation of tablet parameters

Tablets		**Mass (g)**	**Diameter (mm)**	**Height (mm)**	**Hardness against pressure (N)**	**Disintegration time (s)**
Fresh		0.4060 (SD=±0.003)	12.10 (SD=±0.060)	3.50 (SD=±0.023)	93.4 (SD=±2.67)	86 (SD=±27)
Stored at 25 ºC and 60% RH for 4 weeks	**open**	0.4097 (SD=±0.004)	12.13 (SD=±0.050)	3.55 (SD=±0.032)	70.4 (SD=±3.63)	30 (SD=±13)
	closed	0.4078 (SD=±0.004)	12.12 (SD=±0.054)	3.51 (SD=±0.027)	83.6 (SD=±3.37)	54 (SD=±13)
Stored at 40 ºC and 75% RH for 4 weeks	**open**	0.4106 (SD=±0.003)	12.24 (SD=±0.038)	3.69 (SD=±0.016)	65.8 (SD=±2.74)	6 (SD=±4)
	closed	0.4060 (SD=±0.003)	12.10 (SD=±0.009)	3.53 (SD=±0.021)	84.8 (SD=±3.79)	79 (SD=±16)

6 PRACTICAL ASPECTS

The amorphization of CLP as a model API was studied, tablets containing amorphous CLP were produced, and the stability of the product was tested. On the basis of our study, the following approaches can be suggested for the preparation of different solid dosage forms with amorphized API. There are two different methods for the preparation of amorphous products. **Figure 19** outlines the general differences between these two procedures. The second way can be defined as 'in-process' amorphization, because the classical technological formulation process is combined with amorphization of the API.

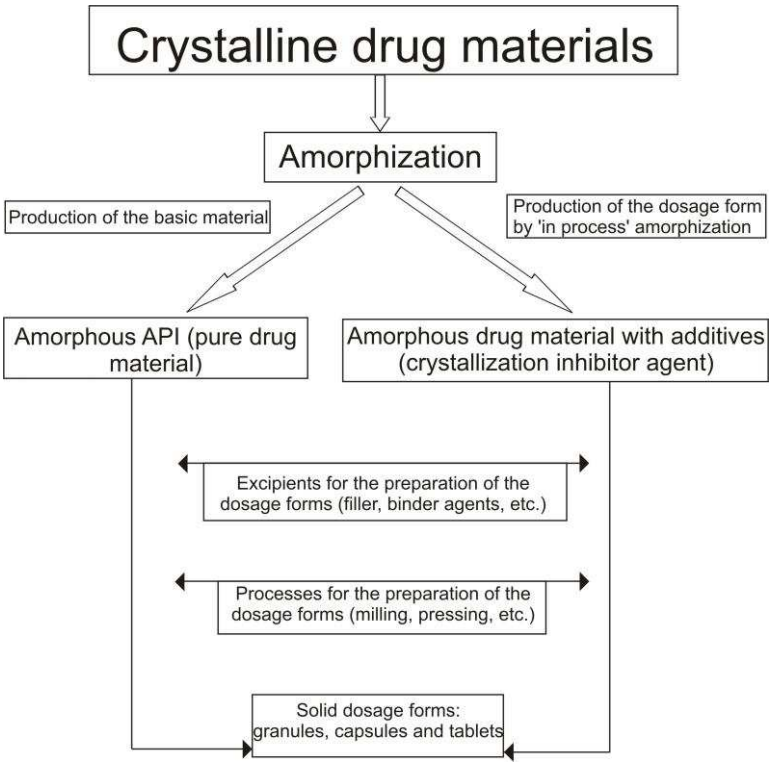

FIGURE 19: Two ways to produce a solid form of an amorphized API

In the light of our results, the following amorphization protocol was developed (**Figure 20**):

- In the first step, a suitable solvent for the API should be selected. In this step, it is very important that the crystalline API should dissolve completely: any crystals remaining in the system can function as seeds and crystallization can start during evaporation of the solvent.

- In the second step, a suitable crystallization inhibitor should be selected and the optimum amount of this auxiliary agent required to maintain the API in amorphous form should be determined. In this step, several crystallization inhibitors should be screened and, if possible, the type of interaction between the API and the auxiliary agent should be investigated.
- In the third step, the amorphization process must be scaled up.
- In the fourth step, the amorphized API should be stabilized on the surface of a carrier. This is an important step in this protocol, because the API, the crystallization inhibitor and the carrier act together in this system to result in stable amorphous API during the technological process.
- In the fifth step, the external phase of the tablets should be mixed with the amorphized product. The mixing must be performed very carefully, because mechanical force can induce recrystallization.
- In the sixth step, the tablets should be pressed. Compression can be a further stabilizing step, fixing the amorphous form because of the smaller surface.

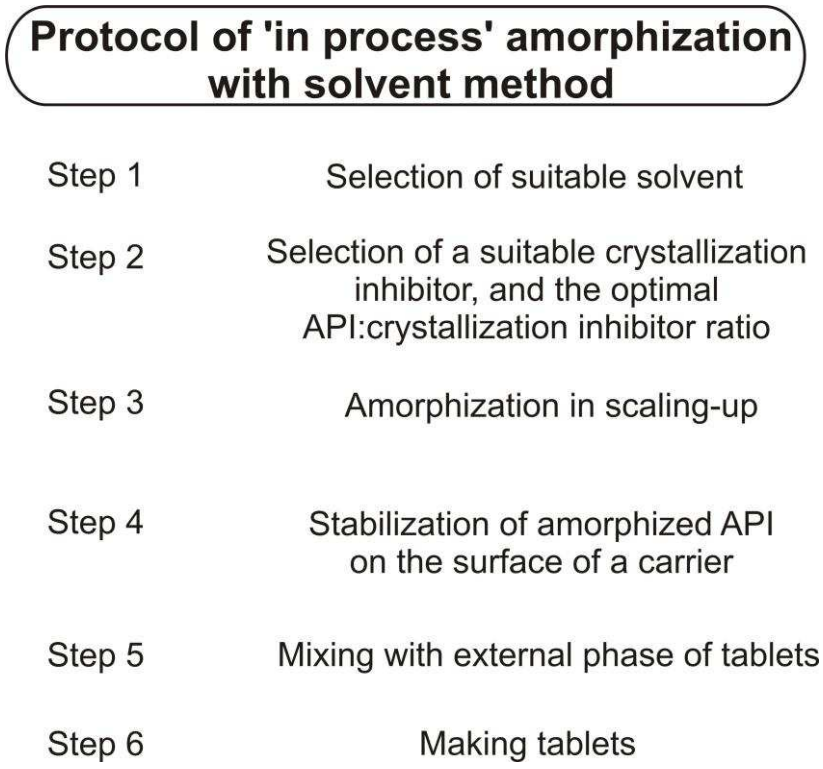

FIGURE 20: Protocol of 'in-process' amorphization.

7 SUMMARY

Our first aim was to investigate the literature background of pharmaceutical amorphization. In this part of this work, the possibilities and the methods of glass formulation were assembled. Many examples were collected that related to solvent, hot-melt and milling technology. Some very well-known, frequently amorphized APIs appeared in all methods. For example, the amorphous form of indomethacin is one of the most widely investigated active agents in this field. Materials were categorized into 2 groups as concerns their amorphization ability: poor and good glass-formers. The methods of solid-state analysis with which glassy materials can be measured were considered.

In the experimental part, CLP was the model API applied in the amorphization. First, we characterized the glassy property of this API. For this, it was necessary to produce a pure amorphous form of CLP without any auxiliary agent. Solvent technology was applied and we chose the most suitable solvent for the procedure. DSC measurement was the appropriate method through which to characterize the T_m and T_g of CLP. The quotient T_g/T_m of CLP was 0.80, and accordingly this API can be classified as a good glass-former.

In the following step, preliminary stability testing was performed. We tested pure glassy CLP from the aspect of recrystallization. We found that after about 1 month recrystallization started, a crystalline fraction appearing in amorphous samples. Because of this observation, the following step of the development was to identify a suitable crystallization inhibitor.

A wide range of auxiliary materials were tested as crystallization inhibitors. These materials can be classified as crystalline, semicrystalline and amorphous agents. The DSC investigations suggested that Aerosil 200, as amorphous hydrophilic SiO_2, was fixed and stabilized CLP in glassy form. The gelling property of Aerosil 200 in ethanol helped in the stabilizaton of the product. We confirmed the presence of H-bonding between CLP and the silanol groups of Aerosil 200 by FT-IR analysis. This secondary bonding means further stabilization of the amorphous product. We then tested the required amount of the crystallization inhibitor. The API:auxiliary material ratio chosen was 7:3, and in the subsequent studies this ratio was applied.

In the scaling-up process, this amorphous product was fixed on the surface of MCC, as a carrier. This step involved a 28-fold scaling-up. We used two different preparation methods.

The better method from the aspects of both the yield and the stability of the product was when Aerosil 200 was in an alcoholic solution of CLP and gave a gel in it was.

We compressed this intermediate product into tablets with suitable auxiliary agents (as the external phase of the tablets) in another scaling-up. We produced 1000 tablets and tested them fresh and after storage. We observed that the final dosage form exhibited better stability from the aspect of recrystallization. This was attributed to the decrease in the surface area of the amorphous product.

Finally, summarizing our results and experience, we devised two amorphization protocol procedures:

- production of a basic pure amorphous product without additives, and
- production of a final dosage form containing amorphous API with ‘in-process’ amorphization.

We introduces the scientific term ‘in-process’ amorphization, into the literature. The general steps were presented in a summarizing figure and can potentially be applied successfully in both scientific and industrial fields.

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ANNEX

I.

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Amorfizálás a gyógyszer-technológiában

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Summary

Révész, P., Laczkovich, O. and Erős, I.: ***Amorphization in pharmaceutical technology***

The amorphization of crystalline active ingredients may be necessary because of the polymorphism of the active substance, the poor water-solubility of the drug material, difficult processing in the crystalline form and the taking out of a patent for a new (amorphous) form. This article introduces protocols for amorphization, which use methods traditionally applied in pharmaceutical technology. The protocols involve three possible routes: solvent methods, hot-melt technologies and milling procedures. With this presentation, the authors suggest help for practising experts to find the correct amorphization method.

Összefoglalás

Kristályos hatóanyagok amorf formába hozatalát indokolhatja a hatóanyag polimorfiára való hajlama, a farmakon rossz vízoldékonysága, kristályos formában történő nehéz feldolgozhatósága valamint egy új (amorf) forma szabadalmaztatása. Az összeállítás bemutat egy amorfizálási protokollt, amely a gyógyszer-technológiában hagyományosan használt módszereket vonultatja fel. A protokoll három lehetséges útvonalat jelöl meg: az oldószeres eljárásokat, az olvadék technológiákat és az őrléses módszereket. A szerzők ezzel az összeállítással segíteni szeretnék a gyakorló szakembereket a helyes amorfizálási technológia kiválasztásában.

Bevezetés

Az amorf forma létjogosultsága közismert, hiszen széles körben alkalmazzák az ipar különböző területein. Ezek az ipari területek többek között a műanyagipar, a textilgyártás, az élelmiszeripar, a félvezetők előállítása, a kerámiagyártás, az optikailag aktív anyagok (optikai üvegek) előállítása és természetesen a gyógyszeripar is.

Az amorf kifejezés formátlan, alaktalan, rendezetlen térbeli elrendeződésű, nem kristályos anyagot jelent. Egyrészről amorf lehet egy térhálós polimer, másrészről a kristályos anyagok hozhatók amorf, vagy más néven üveges állapotba. A kristályos anyagoknak amorfizálási szempontból két csoportja van: a könnyen üvegesedő anyagok („*good glass formers*") és a nehezen üvegesedő anyagok („*poor glass formers*"). A szerves molekulák általában kifejezett szerkezeti flexibilitással rendelkeznek, ami a kristályosodás visszaszorulását eredményezi, ez pedig könnyebb amorfizálást tesz lehetővé [1].

Az amorf forma gyógyszerészeti alkalmazása

Az amorf forma minden előnyével és hátrányával, valamint az amorfizálás nem ismeretlen a gyógyszerforma fejlesztők előtt. Az utóbbi időben azonban a gyógyszerészetben használatos kristályos anyagok amorfizálása óriási lendületet vett, mivel bizonyos esetekben csak ez a módszer vezethet eredményes gyógyszer-formuláláshoz vagy éppen a gazdaságossági szempontok figyelembevétele (pl. szabadalmi bejelentés) dominál. Általában az alábbi esetekben indokolt a gyógyszeranyagok amorfizálása:

(1) *Polimorfiával rendelkező hatóanyagok.* A különböző polimorf módosulatok, amelyek eltérő fizikai-kémiai tulajdonságokkal rendelkeznek, egymásba alakulhatnak, megváltoztatva ezzel többek között oldékonysági tulajdonságukat, préselhetőségüket és nem utolsó sorban biológiai hatásukat [2]. Az amorf formába hozatal megakadályozhatja az egyes polimorf módosulatok egymásba alakulását.

(2) *Kristályos formában nehezen feldolgozható hatóanyagok.* Az esetek többségében megfigyelhető, hogy az amorf forma sokkal jobban préselhető, mint a kristályos alak, ami elsősorban az amorf forma nagyobb deformációs készségével, s az ebből adódó nagyszámú formazáró kötés létrejöttével magyarázható. Más szempontból, nagy jelentősége van az amorf formának az alacsony olvadáspontú kristályos farmakonok préselése esetén.

(3) *Rossz vízoldékonysággal rendelkező hatóanyagok.* Az amorfizálás az oldékonyság-növelés egyik

módja. Amorf formában jobb a hatóanyagok oldékonysága, mert nincs rácsenergia, ami termodinamikai gátja az oldódásnak, mert molekuláris szinten a lehető legnagyobb mértékben lecsökken a hatóanyag részecskemérete és egy hidrofil karakterű kristályosodást gátló segédanyag alkalmazásával nemcsak az amorf forma stabilizálható, hanem az anyag nedvesedése is fokozható [3].

(4) *Lejárt védettségű hatóanyagok újbóli szabadalmaztatása.* Ez egy gazdasági szempontból jelentős kérdés, hiszen a gyógyszer-technológiai módszerekkel történő amorfizálás új lehetőséget nyújt a hatóanyag amorf formában történő bejelentésére (szabadalmaztatására) és különböző gyógyszerformák fejlesztésére.

Amorfizálási protokollok

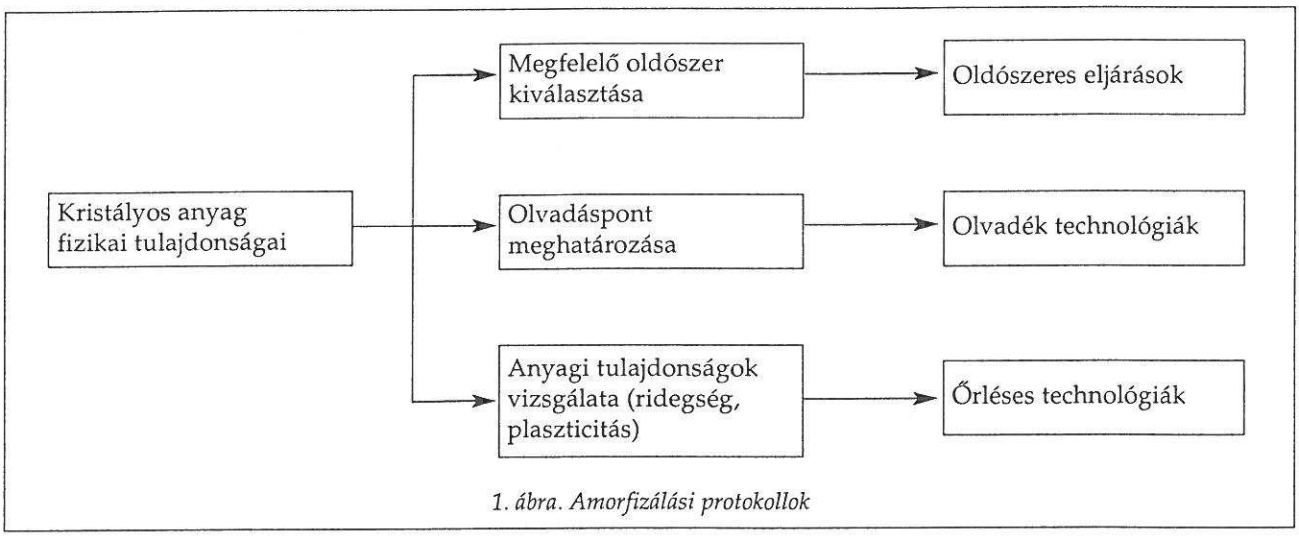
A szerzők célul tűzték ki egy olyan amorfizálási protokoll kidolgozását és bemutatását, amely segítséget nyújthat mindazoknak, akik hasonló céllal kezdenek bele egy munkába. A protokoll összeállítását részben irodalmi adatok, részben pedig saját gyakorlati tapasztalatok segítették.

A teljesség kedvéért meg kell jegyezni, hogy az amorfizálás alapvetően két különböző szinten történhet. Az első esetben maga az alapanyaggyártó amorfizál és eleve amorf formában hozza forgalomba az illető anyagot. Bizonyos anyagok esetében ez jól járható út (pl. őrölt cellulóz), de vannak olyan anyagok amelyeknél stabilitási problémák merülhetnek fel (pl. alfa-laktóz). A hatóanyagok nagy részénél viszont nem is alkalmazható ez a megoldás az azonnali rekrisztallizáció miatt. Ilyen esetben a második szint lép működésbe, azaz a gyógyszerforma fejlesztő amorfizál ún. gyógyszer-technológiai módszerek és segédanyagok (kristályosodást gátlók) alkalmazásával. Mivel egyre nagyobb az igény az amorf forma iránt, így nyugodtan kijelenthető, hogy bizonyos hatóanyagok esetében csak a második szint jelenthet megoldást az amorf forma megjelenítésére és megtartására.

Az amorfizálás kiindulási szempontja a hatóanyag fizikai tulajdonságainak a felderítése. A tulajdonságok alapján három lehetséges útvonalat különböztetünk meg: az oldószeres eljárások, az olvadék technológiák és az őrléses technológiák lehetőségét. Ez a felsorolás egyfajta sorrendiséget is jelent a helyes amorfizálási mód megválasztásában *(1. ábra).*

Oldószeres eljárások

Ebben az esetben központi kérdés az anyag oldékonysága és a megfelelő oldószer kiválasztása. Az oldószer kiválasztásánál feltétlenül figyelembe kell venni az oldószer veszélyességi fokát. Ha csak olyan oldószert találunk a hatóanyag oldatba vitelére, amelyik a 1. veszélyességi osztályba tartozik (pl. szén-tetraklorid), akkor el kell vetni az oldószeres eljárás lehetőségét, és az olvadéktechnológiát kell preferálni. Szerencsés esetben poláris vagy szemipoláris oldószerekkel dolgozhatunk, pl. víz, az etil-alkohol (különböző hígításokban), metil-alkohol stb. Igen fontos szempont, hogy a hatóanyag maradék nélkül feloldódjon az adott oldószerben. Amennyiben kristályok maradnak a rendszerben, úgy azok az oldószer elpárologtatása során, a túltelítési fázisban, oltókristályként beindíthatják a gócképződést. A kitermelés érdekében a hőmérséklet emelésével és koszolvensek alkalmazásával fokozható az anyag oldhatósága. Amennyiben lehetséges, többféle oldószer-



1. ábra. Amorfizálási protokollok

rel célszerű indítani a vizsgálatot, ugyanis vannak oldószerek, amelyek beépülnek a kristályrácsba és elősegítik az üvegesedést.

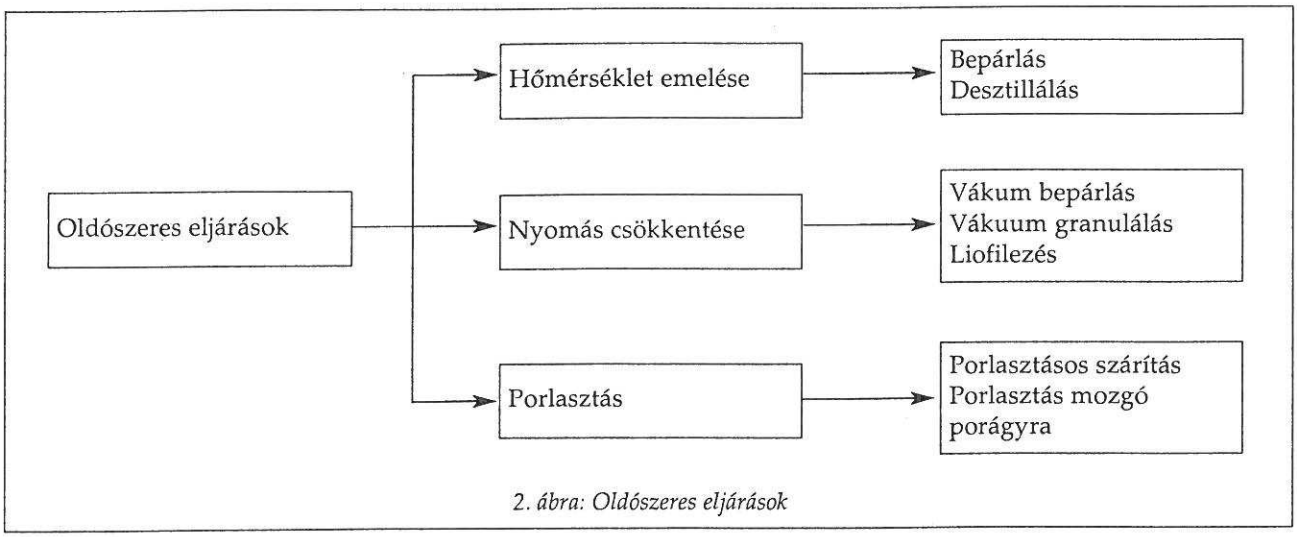
A következő lépés a kiválasztott oldószerrel/oldószerekkel készített oldatból az oldószer *gyors* eltávolítása. A gyorsaság azért fontos, mert ezzel megakadályozható a kristályrácsok felépülése. Ez az oldat a hatóanyagon kívül semmilyen segédanyagot nem tartalmazhat. Az így nyert terméket célszerű azonnal és 24 óra múlva megvizsgálni amorf tartalom szempontjából. Szerencsés esetben az anyagnak van egy „amorfizáló oldószere", amely részben vagy egészében amorf formát eredményez. Kevésbé szerencsés esetben már az azonnali vizsgálattal is a kiindulási kristályokat azonosítjuk. Folytatásként, mindkét esetben javasolható kristályosodást gátló segédanyagok alkalmazása, amelyek nemcsak az amorf forma kialakítását, de annak megtartását is segíthetik.

Az oldószer eltávolítására három lehetőség javasolható (2. *ábra*).

Az első a hőmérséklet emelésével történő oldószer elpárologtatás. Itt is (ahogyan az olvadéktechnológiáknál is) figyelembe kell venni, hogy milyen magas hőmérsékletet visel el a hatóanyag bomlás nélkül. Javasolható a bepárlás és a desztillálás pl. rotációs desztillálók alkalmazásával. Ezek az eljárások elsősorban a termékfejlesztés fázisában javasolhatók.

A második lehetőség a nyomás csökkentése által valósítható meg. Itt technológiai megoldásként a vákuum bepárlás, a vákuum granulálás és a liofilezés (vákuum szublimálás) említendő meg. A liofilezés biztosan amorf formát eredményez, mivel a jég szublimáltatása a rendszerből igen gyors oldószer-eltávolítást jelent. Alkalmazásának egyrészt az szab gátat, hogy csak akkor alkalmazható, ha az oldóközeg desztillált víz vagy híg vizes oldata az etil-alkoholnak, másrészt pedig csupán kis mennyiségű amorf anyag előállítására alkalmas, tehát nem használható ipari méretű amorfizálásra. A módszer azonban nem elhanyagolandó, hiszen amorf referencia-anyag előállítására rendkívül jól felhasználható.

A harmadik út a porlasztásos megoldás. Ez a módszer már nagyobb mennyiségű amorf anyag előállítására is lehetőséget nyújt. Az oldószer ennek a módszernek is akadálya lehet, hiszen ha gyúlékony, porlasztása nehézségekbe ütközhet. A hatóanyag oldatának porlasztása alapvetően kétféle módon történhet. Az egyik lehetőség a porlasztásos szárítás *(spray drying)*, amely gyorsaságából adódóan biztosan amorf formát eredményez. A segédanyagot nem tartalmazó porlasztva szárított termék kiváló referencia anyag lehet. A termék stabilitását azonban célszerű kristályosodást gátló anyagokkal biztosítani. Ezek lehetnek olyan térhálós szerkezetű makromolekulás anyagok, amelyek a hatóanyaggal egy időben oldatba vihetők, de lehet olyan maganyag is, amelyet szuszpendálunk a hatóanyag oldatában és porlasztás során a maganyag felületi sajátságai segítik az amorf forma megtartását. Ez utóbbi megoldás különösen akkor előnyös, ha a termék további feldolgozása szempontjából (pl. tablettázás) annak folyási paraméterei meghatározóak. A porlasztásos technológia másik lehetősége a mozgó porágyra történő oldat felvitel pl. fluidizációs eljárás alkalmazásával. A porágy szerepét töltő- és/vagy kötőanyag egyaránt betöltheti, attól függően, hogy milyen gyógyszerformát kívánunk előállítani az amorfizált termékből. Erre a célra kiválóan alkalmas pl. az őrölt vagy mikrokristályos cellulóz. A módszer jellegéből adódóan feltétlenül javasolt kristályosodást gátló



2. ábra: Oldószeres eljárások

segédanyag alkalmazása. A biztosabb amorfizálás érdekében, amennyiben lehetőség van rá, a segédanyagot is oldjuk fel a hatóanyag oldatában, s ezt porlasszuk rá a maganyagra. Célt érhetünk el abban az esetben is, ha a maganyaghoz keverjük szilárd állapotban a kristályosodást gátló segédanyagot, s az így nyert porkeverékre porlasztjuk rá a hatóanyag oldatát [4–7].

Olvadék-technológa (Hot-melt technology)

Ennél a módszernél elsőként meg kell határozni az olvadáspontot és a bomlás nélkül tolerálható maximális hőmérsékletet. Ezek után két lehetőség adódik. Az elsőnek feltétele az, hogy a hatóanyag megolvadása ne okozza annak bomlását. Így meg lehet olvasztani az anyagot, majd az olvadék hirtelen dermesztésével kialakítható az amorf forma. A második lehetőség, amikor a segédanyagot olvasztjuk meg, és annak olvadékában oldjuk fel a hatóanyagot. Itt is, akárcsak az oldószeres módszernél, elengedhetetlen a hatóanyag tökéletes oldása. Szükség esetén felületaktív anyagok alkalmazása is indokolt lehet. Az olvadék hirtelen dermesztése, valamint kristályosodást gátló segédanyagok alkalmazása ebben az esetben is igen fontos az amorf forma előállítása és stabilitása szempontjából. Nagyobb mennyiségű termék előállítására, gyógyszer-technológiai módszerként, az olvadék extrudálása és a cseppben történő olvadékdermesztés javasolható (*3. ábra*) [4, 7–10].

Őrléses technológia (Milling technology)

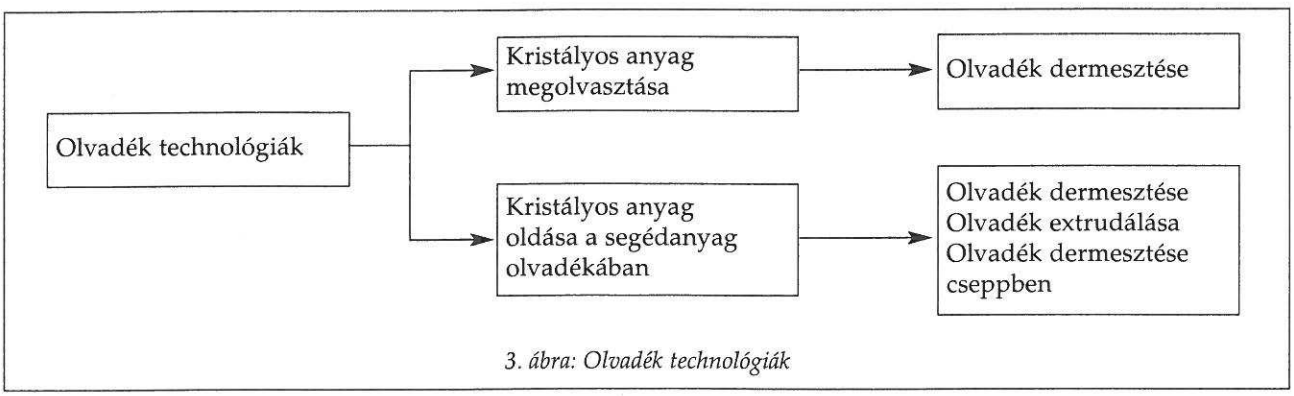
Az őrléses technológia elméleti alapja abban keresendő, hogy külső behatásra a kristályt összetartó erő (kohéziós erő) ellenállást fejt ki az alakváltoztató erőkkel szemben. A rugalmassági határon túl azonban a kristályrácsban maradandó elmozdulás következik be, ami a kristályos állapot megszűnéséhez vezethet. Ez lehet reverzibilis, de lehet irreverzibilis változás is. A rekrisztallizációt ebben az esetben is megakadályozhatjuk segédanyagok alkalmazásával.

Az őrléses technológia kiindulási lépése a farmakon anyagszerkezeti tulajdonságainak megismerése. Közismert, hogy minél ridegebb, törékenyebb egy anyag vagy sok kristályhibával rendelkezik, annál inkább eredményes lehet az őrléses módszer. Az anyagok keménységi értéke, amelyhez a *Mohs*-féle keménységi skála nyújt segítséget, szintén fontos információ a megfelelő őrlő berendezés kiválasztásához [11].

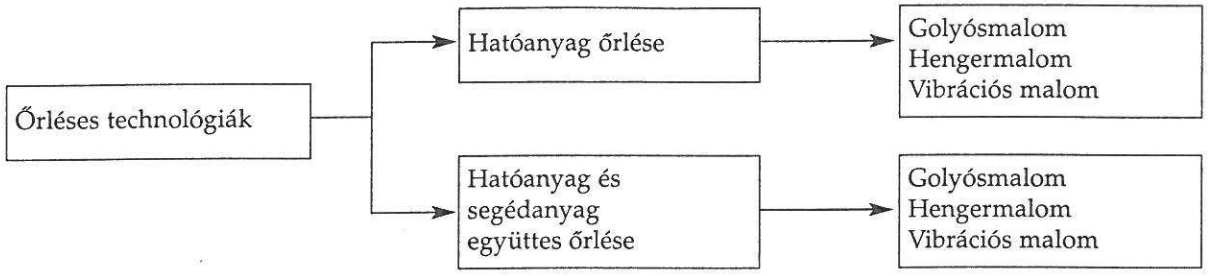
Az egyszerűbb megoldások közé tartozik a golyós-, illetve a hengermalomban történő őrlés, azonban lehetőség van vibrációs malom alkalmazására is (*4. ábra*). Ez utóbbi, az intenzívebb őrlőhatásnak köszönhetően, lényegesen lerövidíti az őrlés időtartamát. Itt is két lehetőség adott. Egyrészt őrölhető a hatóanyag önmagában, másrészt a hatóanyag és a segédanyag együttes őrlése is eredményes lehet. Ez a módszer szilárd diszperziót/oldatot eredményez. Elvi alapja rokon az olvadék-technológiákkal. Az őrlés hatására történő energiaközlés nemcsak a kristályrács felszakadását okozhatja, de helyi olvadást is előidézhet a rendszerben. Kíméletesebb hőterhelést jelent, mint az olvadék-technológiák módszere [12–15].

Stabilitás

Mint ismeretes, az amorf állapot egy magasabb energiaszintet jelent, mint a megfelelő kristályos forma. Ezért a termodinamika törvényeinek megfelelően az amorf anyagok önmagukban rövidebb vagy hosszabb idő alatt visszakristályosodnak. Stabilitásuk fokozása érdekében a következő lehetőségeink vannak: megelőzni a kristályosodást segédanyagok segítségével (pl. polivinil-pirrolidon,



3. ábra: Olvadék technológiák



4. ábra: Őrléses technológiák

cellulóz-éterek, cukrok, cukoralkoholok, szilíciumdioxid származékok), megelőzni a kémiai bomlást és a mikrobák elszaporodását (antioxidánsok, pufferek, tartósítószerek) [1] és megfelelő tárolási körülmények választása, annak érdekében, hogy a lejárati idő megfelelően hosszú lehessen.

Az amorf forma vizsgálata

Míg a kristályos anyagok legjellemzőbb tulajdonsága az olvadáspont, addig az amorf forma egyik legjellemzőbb paramétere az üvegesedési hőmérséklet, rövidítve T_g (*„glass transition temperature"*). Az amorf anyagok nem olvadnak meg, hanem ezen hőmérséklet alatt kemények és törékenyek (akárcsak az üveg), fölötte pedig meglágyulnak. Egy amorf anyag több T_g értékkel is rendelkezhet. A T_g értékének kiszámítására vonatkozó matematikai összefüggés ismert, meghatározására kísérletes úton pedig a DSC (*Differential Scanning Calorimetry*) alkalmas.

A matematikai összefüggés, melynek segítségével megadható a T_g értéke az ún. *Gordon-Taylor* egyenlet [3]:

$$T_g(mix) = \frac{\left[\left(w_1 \cdot T_{g1}\right) + \left(K \cdot w_2 \cdot T_{g2}\right)\right]}{\left[w_1 + \left(K \cdot w_2\right)\right]},$$

ahol $K = \frac{\rho_1 \cdot T_{g1}}{\rho_2 \cdot T_{g2}}$, w= a komponensek tömege, T_g= a komponensek üvegesedési hőmérséklete és ρ = amorf sűrűség.

A DSC vizsgálat minden entalpia-változással járó szerkezeti átalakulás jelzésére alkalmas. A felvételeken általában „lépcsőként" jelenik meg az üvegesedési hőmérséklet.

A T_g meghatározására empirikus úton is lehetőség van. Egyes irodalmi adatok szerint 2/3-a, 4/5-e a megfelelő kristály olvadáspontjának K-ben kifejezve [1]. Más irodalmi adat szerint a megfelelő kristály olvadáspontjának 0,7-szerese K-ben kifejezve [3].

A DSC vizsgálat mellett az amorf forma szerkezeti vizsgálatára még a röntgen diffrakciós vizsgálatokat (X-Ray), a Furier Transzformációs Infravörös Spektroszkópiát (FT-IR), a Közeli Infravörös Spektroszkópiát (NIR) valamint a *Solid State Nuclear Magnetic Resonance* (SSNMR) vizsgálatot használják ellenőrzési céllal.

Amorfizált hatóanyagok, amorfizálási módszerek

Az irodalomban számos hatóanyag fellelhető, melyeket már valamilyen módszerrel amorfizáltak. Az előzőekben bemutatott amorfizálási protokoll három útvonalát követve a *I. táblázat* tartalmaz néhány konkrét példát ezen hatóanyagok köréből a megfelelő irodalmi hivatkozások feltüntetésével együtt. A táblázatban bemutatott példák is igazolják a gyógyszer-technológiai módszerek létjogosultságát a kristályos hatóanyagok amorfizálásában. Remélhetőleg az itt bemutatott eljárások új utakat, lehetőségeket jelentenek a különböző gyógyszerformák fejlesztése területén.

Köszönetnyilvánítás

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I. táblázat

Amorfizált hatóanyagok, amorfizálási módszerek

A módszer elvi alapja	Gyógyszer-technológiai megoldás	Amorfizált hatóanyag	Irodalmi hivatkozás
Oldószeres eljárás	Melegítés	UC-781 (antivirális szer)	*F. Damian, N. Blaton, R. Kinget, G. Van den Mooter:* Int. J. Pharm. 244 (2002) 87-98
	Vákuum	Probucol	*E. Broman, C. Khoo, L. S. Taylor:* Int J. Pharm. 222 (2001) 139-151
	Porlasztás	Nifedipin	*F. Cilurzo, P. Minghetti, A. Casiraghi, L. Montanari:* Int. J. Pharm. 242 (2002) 313-317
Olvadék technológia	Olvadék hirtelen dermesztése segédanyag nélkül („quench cooled")	Itraconazol, Miconazol	*K. Six, G. Verreck, J. Peeters, P. Augustijns, R. Kinget, G. Van der Mooter:* Int. J. Pharm. 213 (2001) 163-173
	Olvadék hirtelen dermesztése segédanyaggal	Indomethacin	*A. Fini, L. Rodriguez, C. Cavallari, B. Albertini, N. Passerini: Int.* J. Pharm. 247 (2002) 11-22
Őrléses technológia	Őrlés segédanyag nélkül	Glisentide	*P. Mura, M. Cirri, M. T. Faucci, J. M. Ginès-Dorado, G. P. Bettinetti:* J. Pharm. Biom. Anal. 30 (2002) 227-237
	Őrlés segédanyaggal	Griseofulvin	*M. Saito, T. Ugajin, Y. Nozawa, Y. Sadzuka, A. Miyagishima, T. Sonobe:* Int. J. Pharm. 249 (2002) 71-79

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[Érkezett: 2004. január 30.]

II.

on diffusion using experimental design for constructing and evaluating the investigations.

Result: Diffusion of the active agent using Franz-type cell is determined by several parameters. The size and the size distribution of cochleates is extremely important which influences the dissolution rate. Evaluating the investigated parameters we can establish the relationship between diffusion and the size and the polydispersity index of cochleates.

Conclusion: The preparation methods of cochleates, the ultrasonication of liposomes and after then the dropwise addition of calcium chloride solution can affect the diffusion of the active agent from cochleates. During our experiments we could observe important and process factors that could be used to optimize the diffusion from these lipid based nanostructures.

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O-26

Protocols for amorphization of crystalline solids through the application of pharmaceutical technological processes

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The amorphization of crystalline active ingredients may be necessary because of the polymorphism of the active substance, the poor water-solubility of the drug material, difficult processing in the crystalline form and the taking-out of a patent for a new (amorphous) form. The amorphization process cannot be applied for most pure materials because of stability of the amorphous form (re-crystallization). In this case, the pharmaceutical technological methods with the application of carriers as a crystallization inhibitor agents can be helped the development of the amorphous form.

The aim of this work was the development of the protocols for amorphization processes, which are traditionally applied in pharmaceutical technology. The first step in the amorphization is the investigation of the physical properties of the active agent. Depending on the properties, three possible routes can be suggested: the *solvent method*, after the finding of a suitable solvent; *hot-melt technology*, after determination of the melting point and the maximum temperature that the agent can tolerate without decomposition; and *milling technology*. This listing implies a certain sequence in the choice of the correct amorphization route.

To the development of the protocols, the authors used different methods (granulation, solidification in drop, fluidization, spray-drying, etc.), different crystalline active agents (meloxicam, ibuprofen, niflumic acid, etc.) and some crystallization inhibitors (polymers, carbohydrates, silica derivatives, etc.). The protocols contain the critical points of the processes (evaporation rate of organic solvent, heating and cooling rates of melted materials, etc.).

With this work, the authors would like to help the experts in the practice to find the correct amorphization method.

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O-27

Stabilization of camptothecin in PLGA nanoparticles

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Objective: The purpose of this study was to develop and characterize poly (lactic-*co*-glycolic acid) (PLGA) nanospheres of camptothecin (CPT):hydroxypropyl-beta-cyclodextrin (HP-beta-CD) inclusion complex to stabilize anticancer drug CPT.

Method: Nanospheres were prepared according to the nanoprecipitation method developed by Fessi et al. Pluronic F68 and CPT:HP-beta-CD inclusion complex were accurately weighed and dissolved in deionized water (15 ml). PLGA was dissolved in acetone (5 ml). Organic phase was then added to the aqueous solution under moderate magnetic stirring. Acetone was eliminated by evaporation under reduced pressure. Particle size and zeta potential measurements were performed by Malvern Zetasizer (Malvern Instruments, UK). Scanning Electron Microscope (Jeol-SEM ASID-10, Japan) and Atomic Force Microscope (Q-Scope^{TM} 350Multimode Quesant Instrument, USA) were used to evaluate surface characteristics of resulting nanospheres. Unbound drug in the nanosphere dispersions was separated by centrifugation at 5000 rpm for 15 min. Resulting nanospheres powder was dissolved in dimethyl sulfoxide, then analyzed by HPLC for the encapsulated drug quantity ($r^2 = 0.9999$). Release kinetics of the drug from the nanosphere was determined in 100 ml of isotonic PBS (pH 7.4) containing 0.1% Tween 80 providing sink conditions in a thermostated shaker bath system at 37 °C with the dialysis technique (Spectra/Por Cellulose Ester Membrane MWCO:100,000 Da, Spectrum Labs, Rancho Dominguez, CA). At predetermined time intervals, samples were withdrawn from the system and replaced with equal volume of fresh release medium.

Results: Mean diameter of nanosphere was 187 nm with polydispersity indices below 0.2. Zeta potential value was almost neutral, −0.057 mV. Examination of photographs of the nanospheres revealed that the surface were smooth and spherical. The encapsulation efficiency was about 3%. In vitro release characteristics of the nanospheres showed that PLGA nanospheres provided controlled release and reduced burst effect with a release profile of up to 48 h. Camptothecin was maintained in its stable lacton form for more than 2 days.

Conclusion: CPT has great potential as an anticancer agent provided that problems with both its physiological stability and insolubility can be solved. The acidic microclimate of PLGA nanospheres has been identified to be the main source

III.

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Amorphization of a crystalline active pharmaceutical ingredient and thermoanalytical measurements on this glassy form

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Abstract Amorphization is nowadays a method that is frequently applied in the pharmaceutical industry. The primary aim of this study is to achieve the amorphization of clopidogrel hydrogen sulphate as an active pharmaceutical ingredient (API) with various solvents and to choose the most suitable one. A secondary aim was to determine the glass-transition temperature (T_g) of this API and to classify it as a good or poor glass former. To investigate the amorphous form, differential scanning calorimetry, X-ray powder diffraction, and FT-IR analysis were applied. The melting point (T_m) was 177.4 °C (450.6 K), and T_g was determined to be 88.9 °C (362.1 K). The quotient T_g/T_m was 0.80, and this API was therefore classified as a good glass former.

Keywords Amorphization · Clopidogrel hydrogen sulfate · DSC · FT-IR spectroscopy · Solvent method · T_g · X-ray powder diffraction

Introduction

The two forms of solids are the amorphous and the crystalline forms [1]. The amorphous form is widely applied in industrial fields such as the plastics industry, the textile industry, the food industry, the production of semiconductors, ceramics and optical glasses, and naturally the pharmaceutical industry. The importance of amorphization is currently increasing in consequence of its value in the pharmaceutical industry for various reasons [2]:

- The development of many poorly water-soluble active pharmaceutical ingredients (APIs) [3].
- The polymorphism of APIs, the different forms having different physical and/or chemical properties [4], which can be interconverted and thus display changing solubility, compressibility, and not at least physiological effects.
- The processing of crystalline drug substances lead run into difficulties, whereas the amorphous form can often be treated easily. In many cases, for example, the compressibility of the amorphous form is better than that of the crystalline form [1].
- Economic aspects are also involved when the aim of amorphization is a new patent relating to the amorphous form.

Amorphous forms of APIs have many useful properties. Some of the most important ones: higher water solubility and higher dissolution rate relative to the crystalline forms [5–8], as there is no lattice energy, which is a thermodynamic barrier to dissolution [9]. There are two feasible ways to amorphize crystalline APIs:

- An amorphous API can be produced alone, without additives, but it must be mentioned that amorphous solids generally have lower stability than the corresponding crystals because of the higher energy level [10–12].
- Otherwise, pharmaceutical technologists often have to apply crystallization inhibitors as additives [13–15]. These inhibitors are usually hydrophilic carriers, which can increase the wetting property of APIs too. This mostly results in more stable product.

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Both routes for the amorphization of crystalline APIs can be applied with the solvent method, hot-melt technology and milling technology [16].

The solvent method is one of the simplest processes for amorphization in industry. It is important that the agent must dissolve without leaving a residue. If crystals remain in the system, then these could start nucleation by acting as seeding crystals in the phase of supersaturation during the evaporation of the solvent.

In pharmaceutical systems, amorphous materials are of great importance both as auxiliary agents and as APIs.

Crystalline systems can be characterized by their melting points (T_m), but amorphous materials do not possess this characteristic temperature parameter. They can rather be defined by the glass-transition temperature (T_g), below which amorphous materials are brittle and above which they are in a liquid or rubbery state. The T_g lies in the interval from approximately 2/3 to 4/5 of the T_m (in Kelvin) [2]. For investigations of the amorphous form and T_g, the differential scanning calorimetry (DSC) is a suitable experimental method [17, 18].

Crystalline agents can be divided into poor (or fragile) glass formers and good (or strong) glass formers. In the case of poor or fragile glass formers $T_g/T_m < 0.7$, and in the case of good or strong glass formers $T_g/T_m > 0.7$ [19]. (In an another publications the limit is given as 1.5, but in this case the quotient is T_m/T_g, when $T_m/T_g > 1.5$ the material is good glass former [11, 20].) Good glass formers exhibit minimal molecular mobility changes at T_g, and hence the shift in heat capacity tends to be small.

In this study, the crystalline API which was subjected to amorphization was clopidogrel hydrogen sulfate (clopidogrel bisulfate) (CLP), a potent oral antiplatelet agent often used in the treatment of coronary artery disease, peripheral vascular disease, and cerebrovascular disease as Plavix® or Iscover® [21]. The chemical formula is $C_{16}H_{16}ClNO_2S$ H_2SO_4 and the molecular mass is 419.9. Chemically it is classed among the thiophenes, and its systematic IUPAC name is methyl (+)-(*S*)-alpha-(2-chlorophenyl)-6,7-dihydrothieno[3,2-c]pyridine-5(4H)-acetate sulfate. Six different polymorphic forms and an amorphous form of the drug have been identified, but only forms I and II are used in pharmaceutical formulations [22, 23]. The polymorphic and amorphous forms of this drug are dealt within a number of patents and articles [24–26].

The primary aim of this study is to achieve the amorphization of CLP by the solvent method and to select the most suitable solvent. A further aim is to characterize the amorphous form via thermoanalytical parameters and to classify it as a poor or a good glass former. We additionally carried out preliminary stability testing of the pure amorphous CLP.

Materials and methods

Materials

CLP polymorphic form II (EGIS, Budapest, Hungary) was used as the crystalline API. Ethanol (Merck, Budapest, Hungary), methanol (Merck, Budapest, Hungary), and acetone (Reanal, Budapest, Hungary) were used as solvents.

Methods

Preparation of amorphous form

Amorphous samples were made using ethanol and methanol. 1.00-g CLP was dissolved in 10.00-g ethanol or 4.00-g methanol with magnetic stirrer (Velp® Scientifica, Europe) for 5 min at room temperature. The solvent was evaporated by two methods: with blown room temperature air or by vacuum (Binder, Germany). 1.00-g CLP was treated with 20.00-g acetone with magnetic mixing for 15 min at room temperature and the solvent was then evaporated in vacuum (Binder, Germany). After drying, samples were pulverized in a porcelain mortar with a pestle.

Differential scanning calorimetry

DSC studies were performed with a DSC 821^e instrument (Mettler-Toledo, Switzerland) with samples of approximately 4.8–5.2 mg weighed into non-hermetically sealed aluminum pans. The samples were heated from 25 to 200 °C at heating rate of 5 °C min^{-1}. The instrument was calibrated with the use of indium.

FT-IR analysis

The FT-IR apparatus was an Avatar 330 FT-IR spectrometer (Thermo Nicolet, USA). The sample, with a CLP content of 0.5 mg, was ground and mixed with 150 mg of dry KBr in an agate mortar, and the mixture was then compressed into a disk at 98.1 kN $(cm^2)^{-1}$. Each disk was scanned 64 times at a resolution of 2 cm^{-1} over the wavenumber region 4000–400 cm^{-1}.

X-ray powder diffraction (XRPD)

XRPD was performed with an X-ray Diffractometer Miniflex II (Rigaku, Tokyo, Japan), where the tube anode was Cu with $K\alpha = 1.5405$ Å. The pattern was collected at a tube voltage of 30 kV and a tube current of 15 mA in step scan mode (4° min^{-1}). The instrument was calibrated with Si.

Results and discussion

Confirmation amorphous form

Prepared samples were measured primarily by DSC. With this method, characterization of the amorphous form is possible quickly. The starting material and five prepared samples were tested at first by DSC. These curves are presented in Fig. 1. The crystalline CLP melted at 177.4 °C (450.6 K). The normalized heat capacity change was 83.9 Jg^{-1}. The sample which was treated with acetone remained in the crystalline phase. The melting point of this material was 177.8 °C (450.9 K) and the normalized heat capacity change was 82.6 Jg^{-1}. The samples prepared in ethanol or methanol were transformed to the amorphous form both on drying through blowing with room temperature air and with vacuum. The characteristic melting point disappeared completely from the DSC curves which were straight lines without any enthalpy changes; no T_g could be detected.

Samples were also tested by XRPD measurement. Diffractograms are can be seen in Fig. 2; for clarity, the diffractograms are displaced along the *y* axis. This investigation supported the DSC results throughout. Figure 2 shows that the products prepared with ethanol or methanol, independently of the drying procedure were converted to the amorphous form, because the peaks disappeared from the diffractograms, and the spectra became smooth. The sample treated with acetone remained in the crystalline phase, and the diffractogram of this preparation was the same as that of the crystalline starting material.

To confirm that no degradation occurred in the prepared samples FT-IR analysis was performed (Fig. 3). For clarity, these spectra too have been displaced along *y* axis. Each peak was present in each spectrum, reflecting the presence of the same chemical bonds and no degradation could be detected in the course of these measurements. The amorphous and crystalline materials furnished the same spectra. Thus, as we had anticipated with this method we could not differentiate the crystalline and the amorphous forms, but we confirmed that no degradation happened in samples.

The DSC, the XRPD, and the FT-IR results suggested that the samples prepared with ethanol or methanol were transformed to the amorphous form independently of the drying procedure, but the sample treated with acetone remained in the crystalline form. The acetone is not suitable solvent for amorphizing CLP, but ethanol and methanol have the same amorphizing property in the case

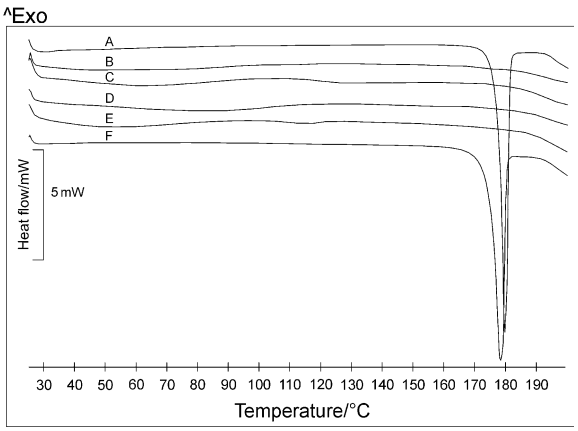

Fig. 1 CLP samples measured by DSC. (*A*) Crystalline form, (*B*) sample prepared with ethanol, dried with blowing of room temperature air, (*C*) sample prepared with ethanol, dried by vacuum, (*D*) sample prepared with methanol, dried with blowing of room temperature air, (*E*) sample prepared with methanol, dried by vacuum, (*F*) sample treated with acetone, dried by vacuum

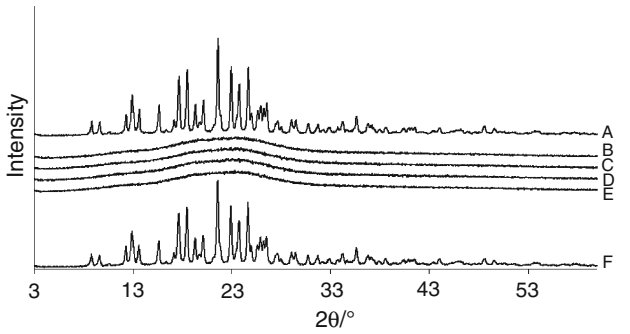

Fig. 2 CLP samples measured by XRPD. (*A*) Crystalline form, (*B*) sample prepared with ethanol, dried with room temperature air, (*C*) sample prepared with ethanol, dried by vacuum, (*D*) sample prepared with methanol, dried with room temperature air, (*E*) sample prepared with methanol, dried by vacuum, (*F*) sample treated with acetone, dried by vacuum

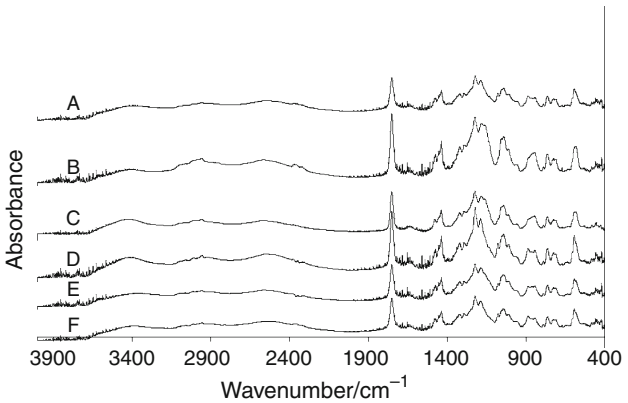

Fig. 3 CLP samples measured by FT-IR. (*A*) Crystalline form (P II), (*B*) sample prepared with ethanol, dried with room temperature air, (*C*) sample prepared with ethanol, dried by vacuum, (*D*) sample prepared with methanol, dried with room temperature air, (*E*) sample prepared with methanol, dried by vacuum, (*F*) sample treated with acetone, dried by vacuum

of this API. If we apply a solvent in a pharmaceutical technological method, we must take care of the danger class of this solvent according to the ICH Q3C guideline [27]. The objective of this guideline is to recommend acceptable amounts for residual solvents in pharmaceuticals for the safety of the patient. ICH Q3C classifies ethanol into the less dangerous Class 3, while methanol is classified into the more dangerous Class 2. According to these facts, we suggest the application of ethanol for amorphizing CLP.

Measurement of T_g

As mentioned in the Introduction, T_g is a very important parameter for amorphous materials. The expected temperature interval, in which T_g can lie, is approximately 2/3 to 4/5 of T_m (in Kelvin) [2]. The T_m of crystalline CLP is 177.4 °C (450.6 K). Accordingly, the expected interval of glass transition is 27.2–87.3 °C (300.4–360.4 K), in this temperature interval we expected the appearance of T_g.

A DSC curve reveals all structural changes accompanied by enthalpy changes. In the curve, T_g is usually indicated by a step, a dislocation from the baseline. For CLP, however, during the first heating run T_g could not be detected, but during the second heating T_g appeared in the curve (Fig. 4). When double heating was carried out with two samples with ethanol, the endothermic step in the DSC curve was detected in the same interval. This temperature interval 82–110 °C (355–383 K) can be defined as glass transition of CLP. The midpoints of these changes were 89.4 °C (362.6 K) and 88.5 °C (361.6 K), with a mean of 88.9 °C (362.1 K). Thus for the calculation this mean of values (88.9 °C = 362.1 K) was applied as T_g. For CLP, therefore the quotient T_g/T_m is 0.80, and accordingly this API can be classified as a good glass former.

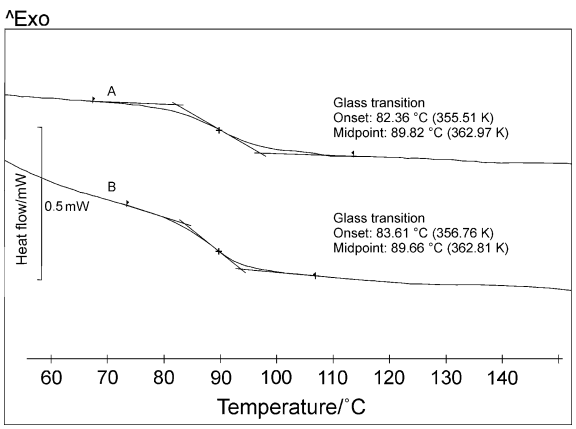

Fig. 4 Glass-transition temperature of CLP measured by DSC. (*A*) Sample prepared with ethanol, dried with room temperature air, (B) sample prepared with ethanol, dried by vacuum

Preliminary stability testing

In the case of amorphous materials, stability problems can occur because of the higher energy level. The possibility of recrystallization is very notable. The amorphous CLP was subjected to preliminary stability testing. A sample prepared with ethanol was stored in a closed glass container at 23 ± 2 °C and 55 ± 5 relative humidity. It was observed that crystal growth started after 30 days (Fig. 5). The crystallinity of the sample increased for approximately 76 days and crystal growth then stopped. The characteristic T_m appeared in curve B at 172 °C (445.2 K). This peak increased linearly with time, but the change stopped after 76 days. T_m was found in the interval of 170–172 °C (443.2–445.2 K). Values of ΔH are given in Table 1. It was clear that recrystallization started in the amorphized sample.

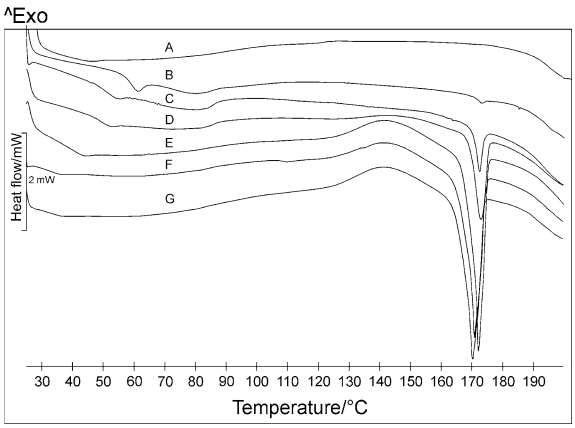

Fig. 5 Preliminary stability testing: (*A*) fresh sample, (*B*) sample stored for 30 days, (*C*) sample stored for 34 days, (*D*) sample stored for 45 days, (*E*) sample stored for 76 days, (*F*) sample stored for 96 days, (*G*): sample stored for 109 days

Table 1 Preliminary stability testing: changes in ΔH determined by DSC measurement

Duration of storage/day	ΔH/Jg^{-1}
0	0.00
30	0.55
34	4.10
45	15.82
76	41.65
96	42.64
109	40.96

Conclusions

In the course of this study, CLP was amorphized by the solvent method. Both ethanol and methanol as solvent resulted in amorphous samples. Either can be a suitable solvent for amorphization, but ethanol is preferred in view of the ICH guidelines relating to residual solvent. Methanol belongs to Class 2, and ethanol in the less dangerous Class 3. In the sample drying procedures, blown room temperature air, and vacuum drying have the same effectiveness. T_g was determined to be 88.9 °C (362.1 K), the quotient T_g/T_m was 0.80, so CLP is a good glass former. The results of the preliminary stability testing indicated that recrystallization occurred in the pure amorphized API after 30 days at room temperature. The application of crystallization inhibitors is therefore suggested.

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IV.

Kristályos vagy amorf forma?

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1. Bevezetés

Az amorf forma a szilárd fázis egyik alfázisának tekinthető a kristályos alfázis mellett[1]. Alkalmazását tekintve az ipar számos területén használják, sőt sok esetben előnyben részesítik a kristályos formával szemben. Alkalmazzák a műanyag gyártásban, a textil iparban, a kerámiák előállítása során, az élelmiszeriparban, az építészetben, félvezetők előállítása során, optikai üvegek készítésekor és természetesen a gyógyszeriparban is. A különböző ipari területek más és más kémiai anyagok amorf formáját alkalmazzák. Három fő csoport nevezhető meg: szervetlen kristályos anyagok amorf formájának felhasználása, szerves kis molekulájú kristályos anyagok amorf formájának használata és nagy molekulájú polimerek amorf formában történő alkalmazása. Ezen összeállítás a gyógyszerészetben használt kristályos hatóanyagok amorf formájának előállításával, tulajdonságaival foglalkozik.

Az amorf forma gyógyszeripari előállítása esetén két nagy csoport különíthető el. Az egyik amikor kristályos anyagok amorf formáját előállítva tisztán amorf formában kerülnek alkalmazásra, illetve további feldolgozásra. Ekkor az amorfizálás elsősorban az alapanyaggyártók által kivitelezett művelet. A másik lehetőség, amikor kristályosodást gátló segédanyagok segítségével amorf rendszereket állítanak elő elsősorban a gyógyszertechnológusok. Ez a két terület jól elkülöníthető egymástól, hiszen az amorf terméket tekintve teljesen más rendszerek állíthatók elő a megjelölt módokon.

Szerkezetüket tekintve az amorf anyagok nem rendelkeznek kristályráccsal. Molekuláik között a rövid távú rendezettség fennáll, de a hosszú távú rendezettség nem alakul ki. A kristályos formával összehasonlítva sokkal energiagazdagabb állapotnak tekinthető[2]. Ebből adódóan stabilitási problémák lépnek fel ezekben az anyagokban. A rekrisztallizáció különböző behatások következtében, vagy egyszerű tárolás során is megindulhat a mintákban[3]. Az amorf anyagok általában higroszkóposak, nagyobb oldódási sebességgel és jobb vízoldékonysággal rendelkeznek, mint a megfelelő kristályok[4].

A gyógyszergyártásban négy területen jön számításba kristályos hatóanyagok amorf formájának alkalmazása[2].

1. A gyakorlatban számos rossz vízoldékonysággal rendelkező hatóanyag kerül alkalmazásra. Az új hatóanyagok egyre gyakrabban sorolhatók a BCS (Biopharmaceutics Classification System) II. vagy IV. osztályba. A rossz vízoldékonyág maga után vonja a nem kielégítő biológiai hasznosíthatóságot. Az amorf anyagoknak nincs kristályrácsuk, ezáltal nincs rácsenergia sem, amely termodinamikai gátja az oldódásnak. Tehát az amorf anyagok alkalmazásának egyik legfontosabb indikációs területe az oldékonyság-növelés.

2. A gyógyszerészetben alkalmazott hatóanyagok nagy része rendelkezik különböző polimorf módosulatokkal. Ezek a módosulatok a gyógyszer-készítmény formulálása során is egymásba alakulhatnak[5]. A polimorfok különböző fizikai és fizikai-kémiai tulajdonságokkal rendelkeznek. A polimorfok egymásba alakulása mindenképpen kerülendő, mivel ezáltal megváltozhatnak alapvető tulajdonságaik (pl. oldékonyságuk, olvadáspontjuk stb.) és ezekkel együtt feldolgozhatóságuk, illetve biológiai hasznosíthatóságuk is. A polimorfok egymásba alakulásának kivédésére egyik kínálkozó megoldás az amorfizálás művelete.

3. Léteznek olyan kristályos hatóanyagok, amelyek kristályos formában nehezen kezelhetőek, formulálásuk során nehézségek merülnek fel. Ezen anyagok feldolgozhatósága az amorfizálással sok esetben javítható, köszönhetően annak, hogy az amorf forma például jobb préslhetőségi tulajdonsággal rendelkezik a megfelelő kristályos anyaghoz képest.

4. Nagyon fontos indikációs területe az amorfizálásnak egy gazdasági szempontból lényeges kérdés. Ez a terület a szabadalmaztatás, hiszen egy védett hatóanyag új szabadalomként való bejelentése történhet meg amorf formában[6].

2. Az amorf forma jellemzése

A kristályos anyagok termikus jellemzésére használt paraméter az olvadáspont. Az amorf anyagok nem rendelkeznek ezzel a jellemző hőmérséklettel, termikus viselkedésük eltér a kristályos anyaghoz képest. Hőmérsékletük emelése közben nem olvadnak meg, hanem egy üvegesedési átmeneten keresztül meglágyulnak, rugalmassá válnak. Ez egy hőmérsékleti intervallum, amely tartománynak a középértékét üvegesedési hőmérséklet ("glass transition temperature", rövidítése T_g) néven említi az irodalom. A kristályos olvadáspontnak (T_m) és az amorf üvegesedési hőmérsékletnek (T_g) az egymáshoz való viszonyából következtethetünk az adott anyag amorfizálhatósági tulajdonságára. Irodalmi adatok alapján ha a

$$\frac{Tg}{Tm} > 0{,}7\,,$$

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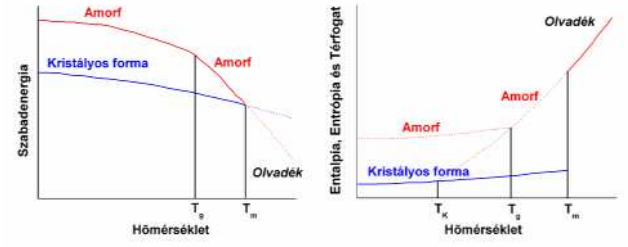
akkor az adott kristályos anyag jól amorfizálható (úgynevezett "good/strong glass former"), ha a

$$\frac{Tg}{Tm} < 0{,}7$$

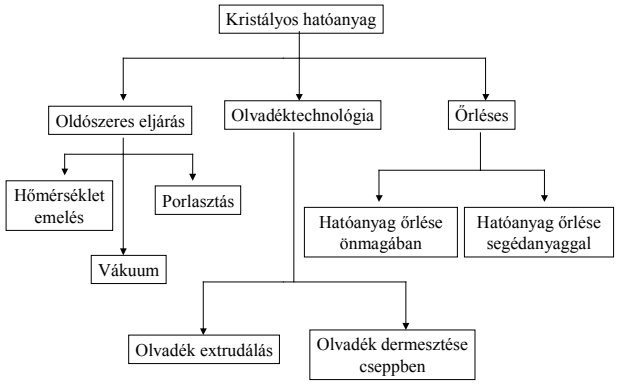
akkor rosszul amorfizálható ("poor/fragile glass former")[2,7]. Más irodalmak a reciprok értéket használják a kategorizálásra, ekkor a határérték az 1,5[7,8]. A T_g érték empírikus tapasztalatok szerint a Kelvinben számított T_m 2/3-a és 4/5-e közötti intervallumba esik[2]. A kristályos anyagokhoz képest az amorf anyagok magasabb szabadenergiával, entalpiával, entrópiával és nagyobb térfogattal rendelkeznek (1. Ábra)[9]. A hőmérséklet emelésével szabadenergiájuk csökken, entalpiájuk, entrópiájuk és térfogatuk növekszik. Az amorf anyag entrópia-változását extrapolálva a kristályos anyag entrópia-változásának görbéjére, megkapható a Kauzmann hőmérséklet (T_K), amely az a hőmérséklet, amelyen az amorf és a kristályos anyag azonos entrópiával rendelkezik. Ha az amorf anyagot a T_K alatt tároljuk, akkor stabilabbnak tekinthető, mint a megfelelő kristályos forma, mivel entrópiája alacsonyabb értéket vesz fel. Ez a jelenség Kauzmann paradox néven honosult meg az irodalomban[6,10].



1. Ábra. Kémiai anyag amorf és kristályos állapotára jellemző hőmérsékletek. T_g: üvegesedési hőmérséklet, T_m: olvadáspont T_K: Kauzmann hőmérséklet[10].

3. Az amorf forma gyógyszeripari előállítási lehetőségei

Az amorf forma előállítására vannak alkalmas gyógyszeriparban hagyományosan alkalmazott módszerek. Alapvetően három módon állítható elő amorf anyag: oldószer segítségével, olvadék technológiával és őrléses eljárással (2. Ábra)[11,12]. Az egyik legkézenfekvőbb a oldószeres módszer. Oldás után az oldószer gyors eltávolítására van szükség, amely megvalósulhat hőmérséklet emelés, nyomás csökkentés és porlasztás segítségével. Az eljárás hátránya a környezet oldószergőzökkel való terhelése, amely probléma megoldására napjainkban egyre nagyobb hangsúlyt fektetnek a gyógyszergyárak. Ezzel szemben az olvadéktechnológia és az őrléses módszer környezet-kímélőbbnek tekinthető lényegesen. Azoknál az eljárásoknál, ahol az anyag hőterhelésnek van kitéve, rendkívül fontos megállapítani az üvegesedési tartományt, hiszen ennek elérése a módszer során mindenképpen kontraindikált.



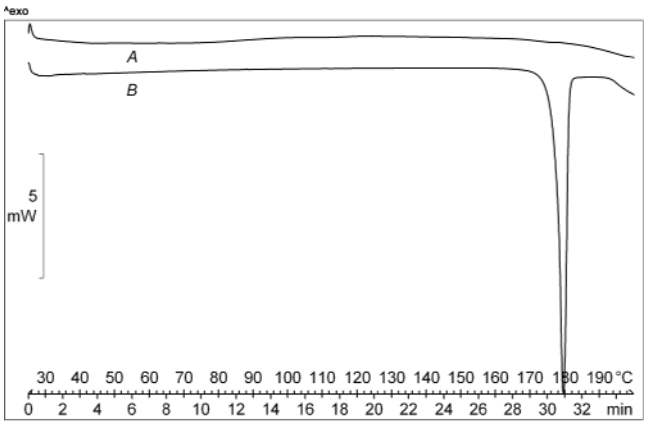
2. Ábra. Az amorf forma gyógyszeripari előállítási lehetőségei

4. Kísérleti rész

A szerzők egy kristályos modell hatóanyag amorf formájának fejlesztését mutatják be, kihangsúlyozva azokat a lépéseket, amelyek elengedhetetlenül fontosak az amorfizálás során.

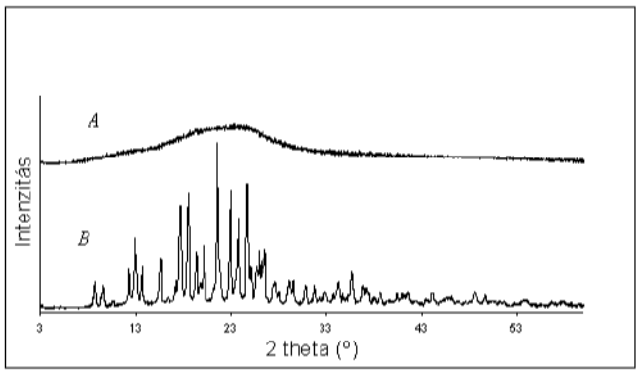
A kristályos vegyületek, mint az elméleti bevezetőben már említésre került, amorfizálhatóság szempontjából két csoportba oszthatók. Annak eldöntésére, hogy a farmakon jól vagy rosszul amorfizálható, a fejlesztés elején mindenképp szükséges egy tisztán amorf minta előállítása és az üvegesedési hőmérséklet meghatározására.

Munkánk során oldószeres eljárással történt meg az amorfizálás. A minták vizsgálata differenciális pásztázó kalorimetria (Differenctial Scanning Calorimetry, DSC, Mettler Toledo DSC 821^e) segítségével valósult meg. Több oldószer kipróbálása után a legmegfelelőbbnek a 96%-os etanolt találtuk. A kristályos anyag feloldása után az oldószer hirtelen elpárologtatása következett szobahőmérsékletű levegő befúvatásával. A mintát, az oldószer elpárolgása után, porítottuk. A minta DSC görbéje a 3. Ábrán látható. Az alsó *(B)* görbe a kristályos mintáról készült felvétel, a felső *(A)* pedig az amorfizált mintáé. A felső görbéről eltűnt az olvadáspont, a minta üveges állapotban került. A T_g nem detektálható.



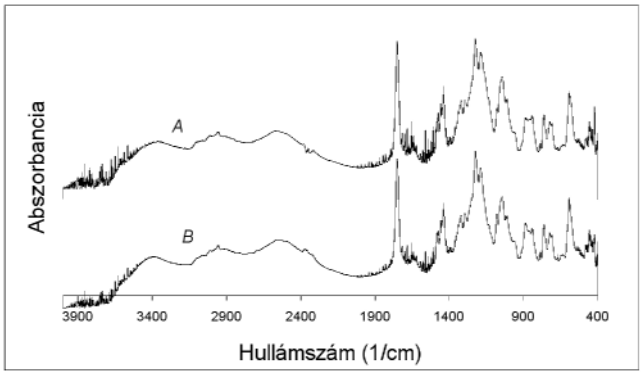
3. Ábra. DSC görbék: A: amorf minta, B: kristályos referencia minta.

Annak bizonyítására, hogy a minta valóban amorf, porröntgen diffrakciós vizsgálatot végeztünk (Rigaku X-ray Diffractometer Miniflex II.). Az eredmények teljesen összhangban állnak a DSC eredményekkel, amely a diffraktogrammokon jól követhető (4. Ábra). Az amorf minta diffraktogrammja kisimult a kristályoshoz képest, a jellemző intenzitás értékek eltűntek, tehát röntgen amorf mintát állítottunk elő.



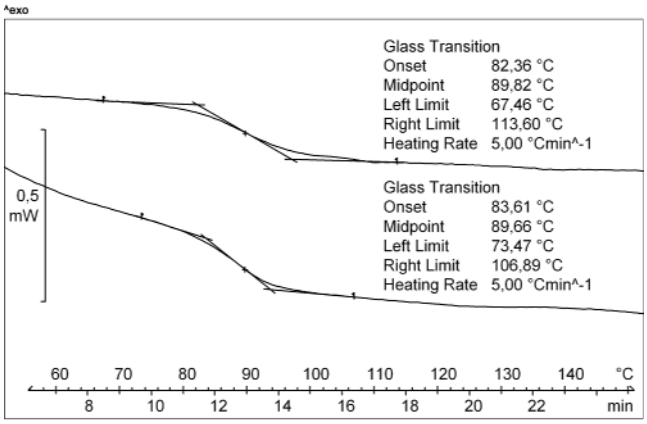
4. Ábra. Porröntgen diffrakciós vizsgálat: A: amorf minta B: kristályos referencia minta.

Annak bizonyítására, hogy nem történt bomlás a mintában és kémiailag azonos molekulákból felépülő anyagot képeztünk Furier transzformációs infravörös spektoszkópiás (FT-IR) vizsgálatot végeztünk (Thermo Nicolet Avatar 330 FT-IR Spectrometer). A spektrumon jól követhető, hogy minden jellemző abszorbancia érték jelen van a görbéken, kémiai átalakulás nem következett be az anyagban (5. Ábra).



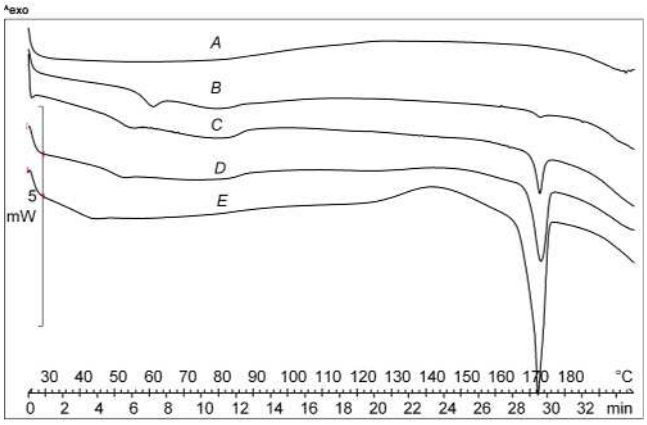
5. Ábra. FT-IR vizsgálat: A: amorf minta spektruma, B: kristályos referencia spektruma.

Az üvegesedési hőmérséklet meghatározására alkalmas módszer a differenciális pásztázó kalorimetria. Első felfűtésre a T_g érték nem volt detektálható. Ugyanazon minta második felfűtése viszont láthatóvá tette az üvegesedési hőmérsékletet (6. Ábra). Kétszer elvégezve ezt a dupla fűtést ugyanabban az intervallumban tapasztaltuk az üvegesedési átmenetet. Az olvadáspontból előre számolt intervallum, amelyen belül várható a T_g 28-89 ºC volt. Az üvegesedési tartomány középértéke 88,9 ºC, mely a T_g értéknek felel meg. Kiszámolva a T_g/T_m hányadost 0,80-ot kaptunk, amely érték alapján a vizsgált anyag a jól amorfizálható hatóanyagok közé sorolható.



6. Ábra. A T_g meghatározása DSC módszer segítségével.

Következő lépésként az amorfizált hatóanyagot előzetes stabilitás vizsgálatnak vetettük alá (7. Ábra). A vizsgálati körülmények: 23±2 ºC és 55±5 % relatív páratartalom voltak. Azt tapasztaltuk, hogy a rekrisztallizáció körülbelül egy hónap tárolás után indul meg. A 7. Ábrán a legfelső görbe, amelyen még nem látható olvadáspont, a frissen előállított amorf anyag görbéje, majd a következő görbék az idő előrehaladtával azt mutatják, hogy a kristályos frakció egyre meghatározóbbá válik a termékben. A karakterisztikus olvadáspontok az idő előrehaladtával egyre növekszenek. Megállapítható tehát, hogy a tiszta amorf anyag nem rendelkezik megfelelő stabilitással.



7. Ábra. A: frissen előállított minta, B: 30 napos tárolt minta, C: 34 napos tárolt minta, D: 45 napos tárolt minta, E: 76 napos tárolt minta.

5. Összefoglalás

Befejezésül megállapítható, hogy az amorfizálásra a gyógyszertechnológiában nagy szükség van elsősorban olyan esetekben, ahol oldékonyság növelése a cél. Az amorf forma számos előnye mellett ki kell hangsúlyozni a stabilitási problémákat is, amely a legnagyobb nehézség ilyen fejlesztések során. A rekrisztallizáció visszaszorítására az egyik lehetőség segédanyagok alkalmazása, de nagyon fontos szerepet kaphat a tárolási

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körülmények helyes megválasztása is. Végezetül a címben felvetett kérdésre az a válasz adható, hogy az amorf forma esetenként előnyben részesíthető a kristályossal szemben, de a fejlesztési folyamat megkezdése előtt mindenképpen meg kell határozni a cikkben felvetett fontos paramétereket, hiszen ezek ismerete nélkül abban sem lehetünk biztosak, hogy amorfizálható-e az adott kristályos farmakon. Ha a hatóanyag a vizsgálatok eredményeként besorolható a jól amorfizálható vegyületek közé, akkor mindenképpen érdemes, egy felmerülő gyógyszeripari probléma megoldására felsorakoztatott lehetőségek közül választani; nem hagyva figyelmen kívül a stabilitási problémákat sem.

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Crystalline or amorphous form

The amorphous form of materials is well known because it is applied in different fields, e.g. the plastics industry, the textile industry, the production of ceramics, the food industry, architecture, the preparation of semi-conductors, the manufacture of optical glasses and naturally the pharmaceutical industry. In pharmaceutical technology the procedures for the production of a glassy form must be divided into two groups: the preparation of the pure amorphous form of crystalline active pharmaceutical ingredients (APIs) and the amorphization of crystalline APIs with a crystallization inhibitor. The amorphization of crystalline APIs may be necessary because of the poor water-solubility of the drug material, the polymorphism of the active substance, difficulty in the processing of the crystalline form, or the taking-out of a patent for a new (amorphous) form. The most important useful property of this form in this field is the increased solubility of drug substances. It should be mentioned that stability problems can occur in the course of application of this form, because of the possibility of recrystallization during the preparation procedure and storage. The aim of this work was to introduce the main properties of the amorphous form, the possibilities of its preparation and the investigation of the developed amorphous API.

For a crystalline model drug the authors determined the quotient T_g/T_m (T_g: glass transition temperature; T_m: melting point), which indicated that this model drug can be classified in the group of good glass formers. Investigation of the stability of the pure amorphous form allowed the conclusion that a crystallization inhibitor should be applied to attain suitable stability.

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RESEARCH ARTICLE

Formulation of tablets containing an 'in-process' amorphized active pharmaceutical ingredient

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Abstract

The aim of this work was a preliminary study of the "in-process" amorphization of clopidogrel hydrogensulfate (CLP) as model drug during the production of tablets as dosage form. A solvent method was used for amorphization and the crystalline phase of CLP was detected by differential scanning calorimetry; the physical parameters of fresh and stored tablets were investigated. For the amorphous form, Aerosil 200 was selected as crystallization inhibitor as the most suitable of eight auxiliary agents. The optimum composition of the product for amorphization in the scaling-up process (100-fold) was 7 parts of CLP to 3 parts of Aerosil 200. In this scaled-up product, the amorphous CLP was fixed on the surface of microcrystalline cellulose. The tablet form further stabilized the amorphous form. Finally, the steps of an "in-process" amorphization are given as a protocol, which can promote stabilization of an amorphized active pharmaceutical ingredient.

Keywords: Aerosil 200, amorphous, clopidogrel hydrogensulfate, crystallization inhibitor, 'in-process' amorphization

Introduction

In pharmaceutical formulations, most drug materials are processed in their crystalline form, which is a thermodynamically stable state that exhibits both short-range and long-range order[1,2]. Unlike a crystalline solid, an amorphous solid has no long-range order of molecular packing, so the molecules are conformationally flexible[3]. The application of an active pharmaceutical ingredient (API) in amorphous form is increasingly common in the development of pharmaceutical solid formulations, with all its risks and benefits[4,5]. The amorphous or glassy form is one of the two solid subphases next to the crystalline form[6]. In pharmaceutical technology, this solid form is well known and widely studied because of its advantageous properties[7,8]. The applications of amorphization can be divided into three groups:

- amorphization of inorganic crystalline materials[9];
- amorphization of organic materials consisting of small molecules (most APIs can be classified in this group)[10]; and
- amorphization of large polymer molecules[11].

The present work is concerned with the second point, using a model API for amorphization.

Amorphization can be applied in pharmaceutical technology for four reasons:

- to increase the dissolution rate and solubility of a poorly water-soluble API[12–15],
- to protect active agents from a polymorphous transformation[16],
- to revise the processability of the corresponding crystalline drug[17], and
- to take out a new patent relating to the amorphous form of a given API (US Patent 6,767,913 B2).

The possibility of amorphization is very important as concerns amorphous formulations, because there are two groups of APIs from the aspect of the glass-forming tendency. Crystalline APIs can be divided into poor (or fragile) glass-formers and good (or strong) glass-formers. There is an empirical formula with which the glass-forming tendency of an API can be predicted. For poor or fragile glass-formers, $T_g/T_m < 0.7$, and for good or strong

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glass-formers, $T_g/T_m > 0.7$, where T_g is the glass transition temperature and T_m is the melting point[18]. If the investigated API can be classified as a good glass-former, its amorphous formulation is possible.

In pharmaceutical formulations, it is necessary to differentiate two possibilities if the chosen API is a good glass-former. The first is when an amorphous form of the API is produced alone, without any auxiliary agents[19,20]. The second is when a composition is made containing both the amorphized API and an auxiliary material(s) as crystallization inhibitor(s). A product made in this way can be a solid dispersion[21] or a solid solution or some other multiple system[22]. Figure 1 outlines general differences between these two ways. The second way can be defined as "in-process" amorphization, because the classical technological formulation process is combined with amorphization of the API. The technologies which are used for making an amorphous form in pharmaceutical technology[23] are a solvent method[10], hot melt technology[24] and a milling process[25].

In this work, clopidogrel hydrogensulfate (clopidogrel bisulfate) (CLP) (methyl (+)-(S)-α-(2-chlorophenyl)-6,7-dihydrothieno[3,2-c]pyridine-5(4H)-acetate sulfate)[26] was used as model API for amorphization. This API is a potent oral antiplatelet drug often used in the treatment of peripheral vascular disease, coronary artery disease and cerebrovascular disease. Six different crystalline forms (I–VI) and an amorphous form of CLP are known in the literature. In pharmaceutical technological formulations and in therapy, polymorphs (Ps) I and II have been used to date[27]. Our preliminary investigations indicated that this API is a good glass-former, with $T_g/T_m = 0.80$, but with a considerable tendency to undergo recrystallization[28].

The aim of this work was to study the "in-process" amorphization of CLP during the production of tablets as dosage form. We report here the selection of the crystallization inhibitor, the amorphization of CLP in the scaling-up process and the stabilization of the amorphous form as regards the composition of the tablets. Finally, the steps of an "in-process" amorphization protocol are given which can promote stabilization of an amorphized API.

Materials

The empirical chemical formula of CLP II (EGIS Company, Hungary) is $C_{16}H_{16}ClNO_2S \cdot H_2SO_4$ with molecular mass 419.9. Chemically, it is classed among the thiophenes, and its systematic IUPAC name is methyl (+)-(S)-α-(2-chlorophenyl)-6,7-dihydrothieno[3,2-c]pyridine-5(4H)-acetate sulfate. The solvent applied was ethyl alcohol 96% v/v (Merck Kft., Hungary). Our previous study showed that this solvent is the most suitable for amorphization of CLP[28]. In this study, we used acetone, methanol and ethanol as solvents. Our results suggested that the samples

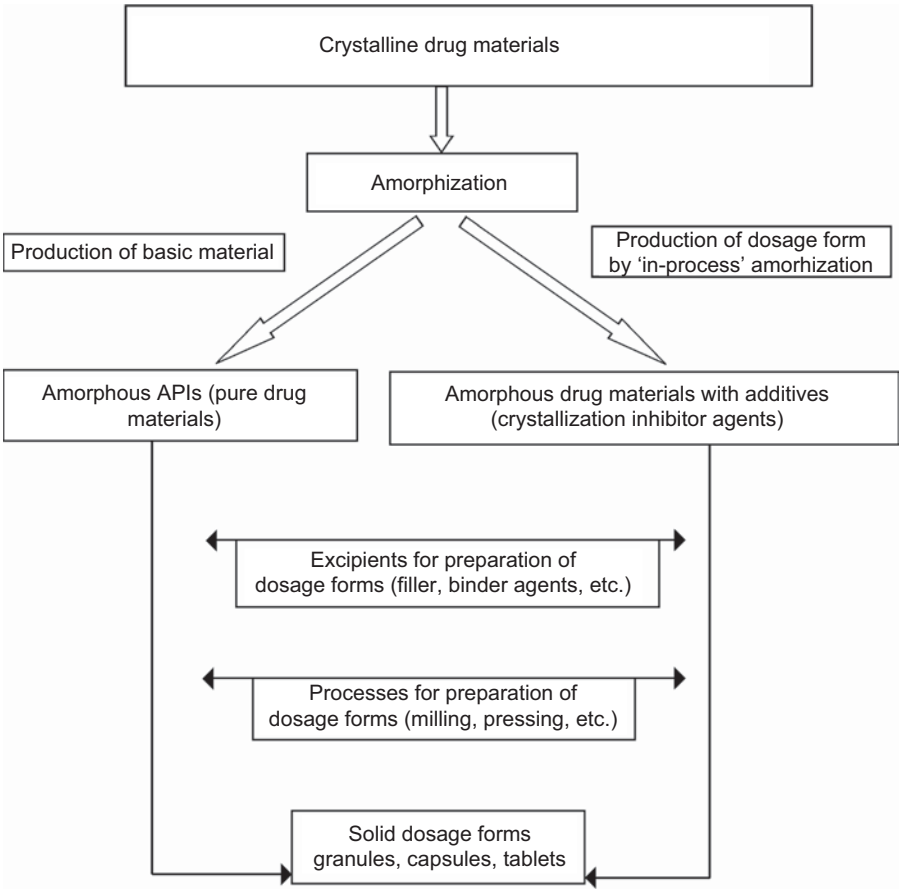

Figure 1. Two ways of making a solid form of an amorphized API.

prepared with ethanol or methanol were transformed to the amorphous form independently of the drying procedure, but the sample treated with acetone remained in the crystalline form. ICH Q3C classifies ethanol into the less dangerous Class 3, while methanol is classified into the more dangerous Class 2[38]. According to these facts, we suggest the application of ethanol for amorphizing of CLP.

As possible crystallization inhibitors, the following excipients were applied: Aerosil 200 (colloidal SiO_2, Nippon Aerosil Co., Japan; with hydrophilic properties), Syloid 72 FP (porous SiO_2, Grace, Hungary, with hydrophilic properties); kaolin (Merck Kft., Hungary); mannitol (Merck Kft., Hungary); microcrystalline cellulose (MCC) (Avicel PH 101, FMC Corporation, Europe); polyvinylpyrrolidone (PVP K25) (PVP, Kollidon® 25, BASF, Germany); methylcellulose (Ph. Eur.) and cross-linked PVP (Crospovidone, Kollidon® CL-M, BASF, Germany) named PVP K CL-M.

MCC was used as filler in tablet making. Cross-linked PVP (PVP Polypl. XL 10) (Polyplasdone® XL 10, N-vinyl-2-pyrrolidone polymer, I.S.P. Technologies Inc., Germany) was applied as disintegrant, and magnesium stearate (Hungaropharma, Budapest, Hungary) as lubricant agent in the composition of the final tablets.

Methods

Preparation of amorphous reference sample

An amorphous reference sample was made by using ethyl alcohol 96% v/v. 1.00 g of CLP was dissolved in 10.00 g of ethyl alcohol with the aid of a magnetic stirrer (Velp® Scientifica, Europe) for 5 min at room temperature. The solvent was evaporated off with blown room-temperature air. After drying, the sample was pulverized with a pestle in a porcelain mortar.

Selection of crystallization inhibitor

Different masses of CLP were dissolved in different amounts of ethyl alcohol 96% v/v. The resulting solutions were mixed with different crystallization inhibitors in a porcelain mortar, heading to solution or suspension or gel formation. The ratio CLP:crystallization inhibitor was 7:3. The mixtures were then dried with room-temperature air (25°C, 46% relative humidity (RH)). After the most suitable inhibitor had been chosen, it was mixed with CLP in ratios of CLP 1:9; 3:7; 1:1; 7:3 and 9:1 with the aim of finding the best active API/auxiliary agent ratio.

Amorphization in scaling-up process

Sample 1: 28.0 g of CLP was dissolved in 160.0 g of ethyl alcohol 96% v/v with the use of a magnetic mixer for 2 min. 12.0 g of Aerosil 200 and 40.0 g of MCC were mixed with a Turbula mixer (speed: 50 rpm, duration of mixing: 5 min). The solution of CLP was then vaporized onto the surface of the Aerosil 200-MCC mixture bed in a pan (Dragex-1, Jørgen).

Sample 2: 28.0 g of CLP was dissolved in 160.0 g of ethyl alcohol 96% v/v with the use of a magnetic mixer for 2 min. 12.0 g of Aerosil 200 was added to the solution of CLP and underwent solvation in 2 min; a gel was made by mixing. This mixture was vaporized onto the surface of 40.0 g of a MCC bed in the same pan.

The parameters (in both cases): pan (Dragex-1 stainless steel equipment), which was furnished with exhauster system for removal of solvent under processing, rotation speed: 25 rpm; pressure of spraying air: 0.1 bar; type of vaporizer: Walther, 1 mm nozzle diameter; drying air temperature: 25°C; RH of drying air: 46%; transportation of liquid: Peripump; speed of transportation: at the beginning of measurement 5 ml/min; at the end of measurement: 1 ml/min (this depends on the speed of drying). This step involves a 28-fold scaling-up. During the process, the loss of powder was very different depending on the place of Aerosil (in powder bed or in alcoholic solution).

Tablet making

A larger amount of stabilized product was prepared with the production method employed for *Sample 2*. This product was the internal phase of the tablets. The mass of a tablet was 400 mg, containing 100 mg of CLP. The composition for 1000 tablets is given in Table 1. The internal and external phases were mixed with a Turbula mixer (speed: 50 rpm, duration of mixing: 5 min). Tablets were made with a Korsch EKO eccentric tablet machine (Emil Korsch Maschinenfabrik, Berlin, Germany) (35 tablets/min, punch diameter 12 mm, compression force: 9 ± 1 kN).

Differential scanning calorimetry

For characterization of the amorphous form, differential scanning calorimetry (DSC) was used (a Mettler-Toledo DSC 821 instrument). Approximately, 4.80–5.20 mg of sample was placed into an aluminum pan which was then sealed and scanned from 25°C to 200°C at 5°C/min under an argon gas flow at 100–150 ml/min.

FT-IR analysis

Confirming the presence of H-bonding in the samples, we used FT-IR analysis in solid and in liquid phase. Dichloromethane was applied as solvent for preparation of solutions. In liquid phase, concentrations were 0.1000 g/10 cm^3, 0.0500 g/10 cm^3, 0.0250 g/10 cm^3, 0.0125 g/10 cm^3. Infrared spectra were recorded on a Furier transform infrared spectrometer (Bio-Rad Digilab Division FTS-65A/869, Philadelphia, PA) between 4000 and 400 cm^{-1}. The spectrometer was equipped with a

Table 1. Composition for 1000 tablets.

	Substances	Mass
Internal phase	CLP	100.00 g
	Aerosil 200	42.86 g
	MCC	243.14 g
External phase	Polypl. XL 10	12.00 g
	Magnesium stearate	2.00 g

DTGS detector for the measurement of solid sample. Solutions were investigated in KBr liquid cell of 0.1 mm thickness. The spectral resolution was 4 cm^{-1} and 128 scans were averaged.

For the chemical stability testing of samples, we used FT-IR apparatus also. The equipment was an Avatar 330 FT-IR spectrometer (Thermo Nicolet, Madison, WI). The sample, with CLP content of 0.5 mg, was mixed with 150 mg of dry κBr in an agate mortar, and the mixture was then compressed into a disc at 10 t. Each disc was scanned 64 times at a resolution of 2 cm^{-1} over the wavenumber region 4000–400 cm^{-1}.

Investigation of tablet parameters

Five parameters of the tablets were investigated: mass, diameter, height (measured with a screw micrometer; Mitutoyo Corporation, Tokyo, Japan), hardness against pressure (Heberlein apparatus, Le Locle, Switzerland) and the time of disintegration (Erweka ZT71, GmbH, Germany). Investigations were made with fresh and with stored tablets.

Investigation of stability of products

As recommended by international guidelines (ICH Q1A), we stored samples under two different conditions. Long-term testing was performed at 25 ± 2°C with 60 ± 5% RH, and accelerated testing at 40 ± 2°C with 75 ± 5% RH. Under both conditions, samples were stored in open and in closed containers; the duration of storage was 4 weeks.

Results and discussion

Selection of crystallization inhibitor

The aim of this investigation was to select a suitable crystallization inhibitor. As mentioned in the Introduction, CLP is a good glass-former. Ethyl alcohol 96% v/v was used as amorphizing solvent for CLP. Amorphous CLP is unstable: its recrystallization starts within a month[28]. The stability of CLP can be increased through the use of a crystallization inhibitor, which is the auxiliary agent in the tablet composition. In this step, different crystallization inhibitors were tested: Aerosil 200, Syloid 72 FP, kaolin, mannitol, MCC, PVP K25, methylcellulose and PVP K CL-M. These auxiliary agents can be classified as crystalline (e.g. mannitol), semicrystalline (e.g. MCC) and amorphous (e.g. Aerosil 200) materials. Their common property is a large specific surface and they undergo physical interactions (secondary bonding) with numerous materials. These properties can prevent the growth of crystals and the development of the long-range order of molecules of APIs.

DSC curves of the reference CLP (crystalline and amorphous) and samples with CLP can be seen in Figure 2. The thermogram of crystalline CLP exhibited a sharp endothermic peak at 177.4°C, corresponding to the melting point of CLP. The scan of the amorphized reference CLP did not contain any characteristic peak, of course. For the sample in which mannitol was present as crystallization inhibitor, the peak occurred at about 146°C, due to the dissolution of CLP in the melted mannitol (T_m for mannitol is 165°C)[29]. The samples with

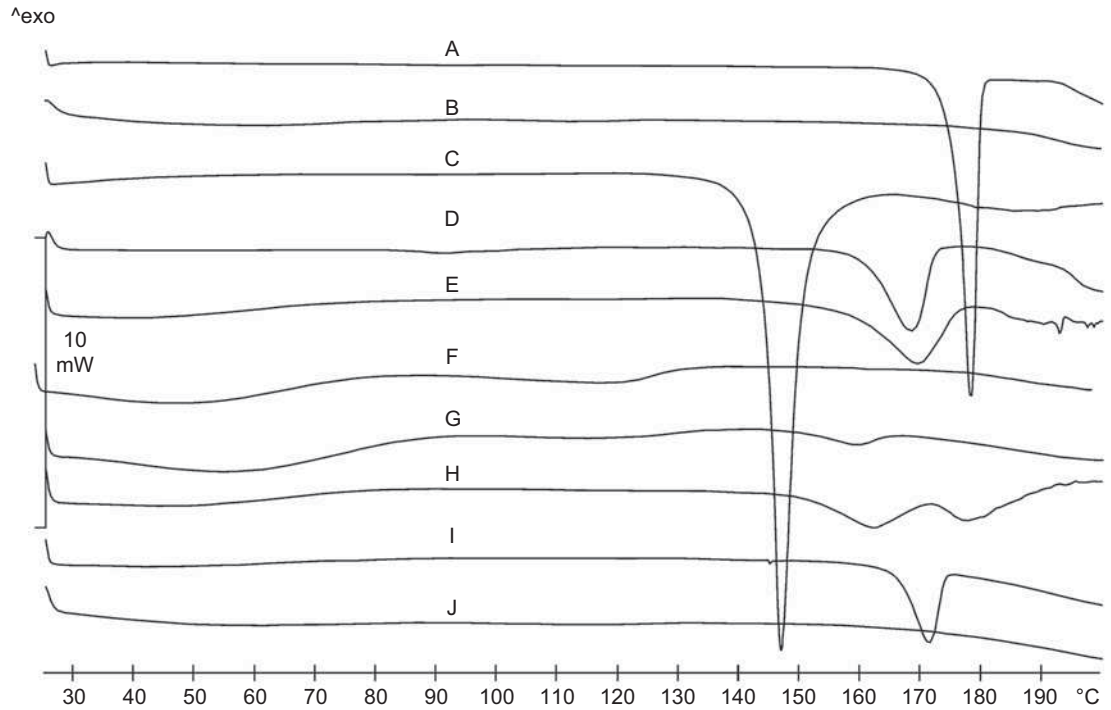

Figure 2. Effects of crystallization inhibitors on crystallinity of CLP. DSC curves of reference materials and samples made with different crystallization inhibitors. **A**: Crystalline reference sample, **B**: amorphous reference sample, **C**: sample with mannitol, **D**: sample with kaolin, **E**: sample with MCC, **F**: sample with PVP K25, **G**: sample with PVP K CL-M, **H**: sample with methylcellulose, **I**: sample with Syloid 72 FP, and **J**: sample with Aerosil 200.

kaolin and MCC exhibited decreased CLP T_m values (168 and 169°C). In this range, these agents do not have melting points. The samples with PVP K25 and PVP K CL-M contained CLP in amorphous form, but suffered a color change because of incompatibility between the components. The curve for the sample made with methylcellulose displayed a double peak effect, at 167.9°C and 177.6°C. This may be an indication of two Ps in this sample. It has been reported that P IV melts at 167.9°C[30] and P II at 177.6°C[31]. Accordingly, this sample contains three different forms of CLP: the amorphous form, and the crystalline forms P IV and P II. Aerosil 200 and Syloid 72 FP differ greatly in applicability as crystallization inhibitors despite both of them consisting of SiO_2. With ethyl alcohol as solvent, Aerosil 200 resulted in perfectly amorphous CLP, in contrast with Syloid 72 FP, which amorphized the CLP only partially. This result can be explained as a consequence of the gelling property of Aerosil 200 in ethyl alcohol. After the evaporation of the ethyl alcohol, the large surface of SiO_2 fixes the CLP and protects against crystal growth, because of the interaction between CLP and SiO_2. This interaction presumably involves H-bonding with the surface silanol groups of SiO_2. Such silanol groups are not present on the surface of Syloid 72 FP, which rules out this interaction. This supposition is based on the reported verification of the presence of H-bonding between indomethacin and SiO_2 by solid-state nuclear magnetic resonance imaging[32]. These results led us to choose Aerosil 200 as crystallization inhibitor for the scaling-up process. This auxiliary agent is a classical additive in pharmaceutical formulations. In the case of solid forms, it can be used as glidant[33] or coating material[34] in tablet making, as surface modifier (for example, DPI formulations, due to its highly hydrophilic and adsorbing property)[35] and as auxiliary agent in the case of preparation of solid dispersion[36]. It is an amorphous agent itself, and thus the presence of API crystals in samples can be detected unambiguously, e.g. by DSC.

The following step in this work was to find the optimum CLP/Aerosil 200 ratio. Five different compositions were tested with this aim. The DSC curves are presented in Figure 3. Melting is not detected in curves **A**, **B**, **C**, **D** and **E**, and these samples can therefore be regarded as amorphous. Curve **F**, which relates to a CLP:Aerosil 200 ratio of 9:1, indicates T_m at 169.5°C, which means the recrystallization of CLP. Accordingly, this amount of crystallization inhibitor is not sufficient to maintain the active agent in amorphous form. For this reason, a CLP:SiO_2 ratio of 7:3 was chosen for tablet formulation.

Amorphization in scaling-up process

The next step was to stabilize amorphous CLP on the surface of the carrier. In this system, MCC was used as the carrier, which serves as a filler/binder in tablet making. In *Sample 1*, the alcoholic solution of CLP was vaporized onto the surface of the mixture of MCC and Aerosil 200. With this preparation procedure, the powder underwent considerable outflow from the pan (powder effusing or dusting). The yield of the preparation was only 64.8%. For *Sample 2*, only MCC was added to the pan. The mixture of CLP and Aerosil 200 was dissolved in ethyl alcohol (96% v/v) and vaporized onto MCC bed. The yield of this preparation method was 85.2%, clearly indicating

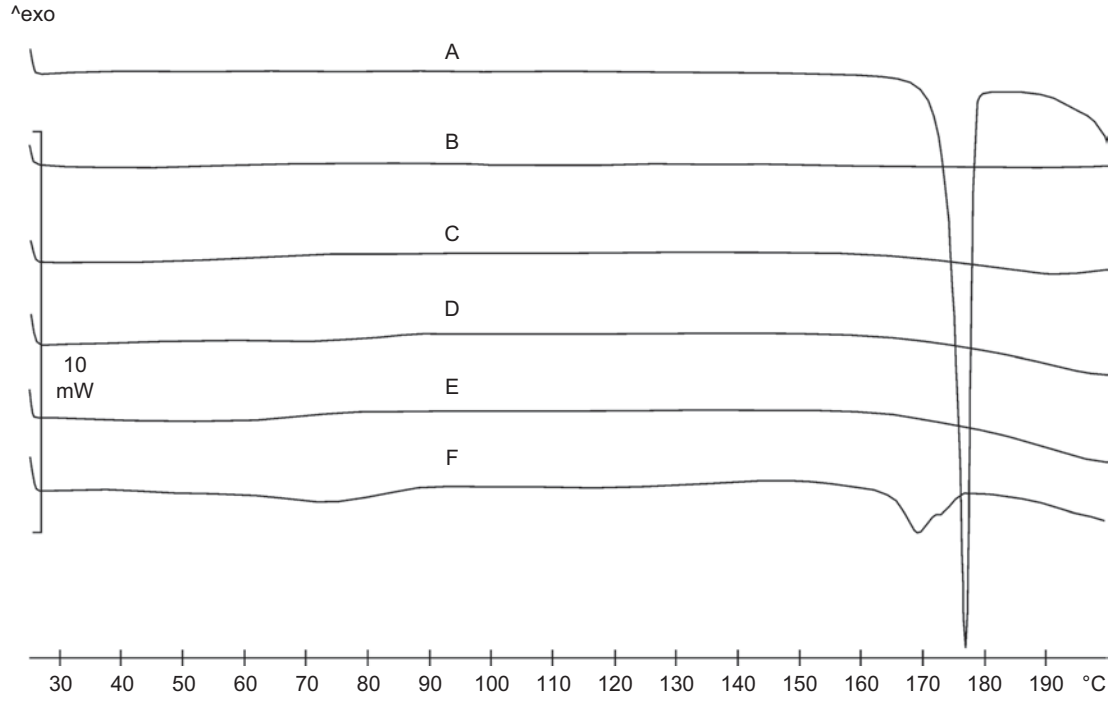

Figure 3. DSC curves of samples containing Aerosil 200. **A**: Reference (crystalline CLP), **B**: CLP:SiO_2 = 1:9, **C**: CLP:SiO_2 = 3:7, **D**: CLP:SiO_2 = 1:1, **E**: CLP:SiO_2 = 7:3, **F**: CLP:SiO_2 = 9:1

that the processing of Aerosil 200 in the liquid phase is more advantageous. The DSC scans of both samples and a physical mixture (Figure 4) demonstrated that the CLP in both samples was in the amorphous form (in contrast with the situation for the physical mixture) because there was no sign of T_m in the curves.

To verify of H-bonding between silanol groups of Aerosil 200 and CLP molecules, we compared FT-IR spectrums of *Sample 2* and the corresponding physical mixture (see Figure 5). In the interval of 900–600 cm^{-1}, spectrum **A** contains vibrations of C structure's deformation. These bands decrease remarkably in spectrum **B**. It refers that the product is in amorphous state. On the other hand, it denotes the presence of a chemical bonding in the product.

To confirm the chemical bond, we measured *Sample 2* in different concentration in solutions (see Figure 6). In the interval of 1100–1000 cm^{-1} spectrum **A** shows wide association. With the dilution of the sample, this association breaks off continually (spectrums **B**, **C**, **D**, **E**). Between 1058 and 1036 cm^{-1}, two bands were appeared that characterize ν_{AS} C-O-C stretching (this marks acetates). The band, at 1058 cm^{-1} shifted to the band, at 1036 cm^{-1} with the dilution. It means that the association breaks off.

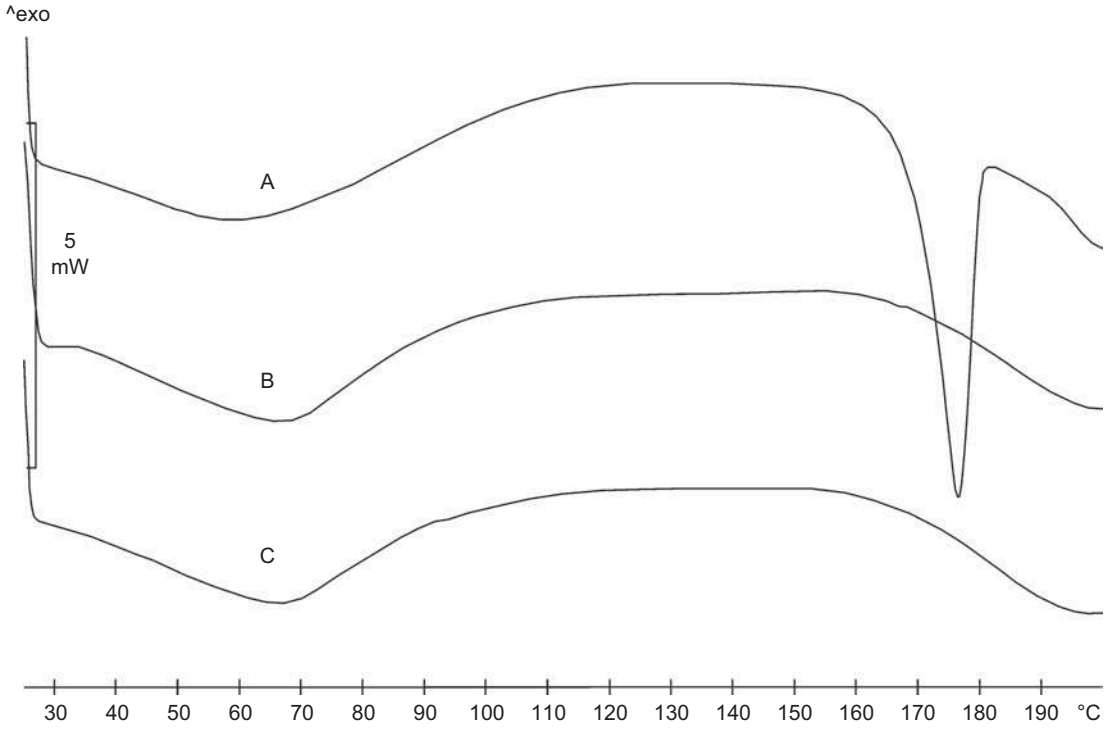

Figure 4. DSC curves of samples. **A**: physical mixture, **B**: *Sample 1*, **C**: *Sample 2*.

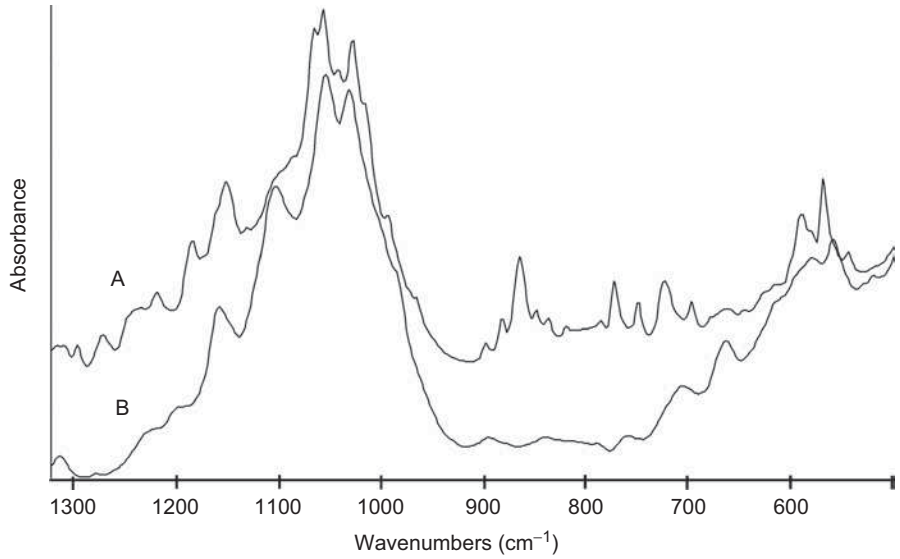

Figure 5. FT-IR analysis of *Sample 2* and the corresponding physical mixture. **A**: physical mixture, **B**: *Sample 2*.

These results justify the presence of H-bonding in the solid *Sample 2*. The different states of samples cause different places of bands. The spectrum **F** shows that dichloromethane has not got sign in this interval.

In the study of the stability of amorphous CLP, *Sample 1* and *Sample 2* were stored for 4 weeks at 25°C and 60% RH. The results revealed that the stored samples remained in the amorphous phase. The findings of accelerated testing showed that when *Sample 1* was stored in either open or closed containers after 4 weeks, it included crystalline material. For *Sample 2*, only the sample stored in an open container included a crystalline phase.

To investigate the chemical stability of *Sample 2*, FT-IR analysis was used. The FT-IR spectrums showed no chemical changes in the samples, which were stored in open and closed containers. All of the peaks are the same in the fresh and in the stored samples (see Figure 7). It can be concluded that the preparation procedure applied for *Sample 2* is better, and this product is more stable than *Sample 1*. In *Sample 2*, the gel structure of the CLP/Aerosil 200/ethyl alcohol system extends the adherence of the CLP/Aerosil 200 system on the surface of MCC. There is an interaction between surface silanol groups of Aerosil 200 and hydroxyl groups of MCC on its surface, which is a hydrogen bonding[37]. This interaction can come into existence easily in liquid phase (*Sample 2*), than in solid phase in the case of a simple mixing (*Sample 1*). For these reasons, the amorphization procedure used for *Sample 2* was applied in tablet making.

Tablet making and investigation of tablets

The amorphization procedure applied for *Sample 2* was used to make 1000 tablets. This tablet composition (see Table 1) resulted in good flowability and tablettability.

Both fresh and stored tablets were investigated. In the thermoanalytical study, a physical mixture of the tablet components was also investigated because of the presence of the crystalline phase of CLP. The DSC curves are depicted in Figure 8. The slight enthalpy changes detected in curve **E** indicate that this sample may contain a little crystalline phase. This sample was stored at 40°C and 75% RH in an open container. Curves **A**, **B**, **C**, **D** and **F** do not reveal any crystalline phase in the system. A feature of importance for tablet making was that the surface area of the amorphous product decreased, which was another stabilizing step in the formulation.

The physical parameters of the fresh and stored tablets are reported in Table 2. The change in mass of the tablets was greatest for the tablets stored in an open container under accelerated conditions (40°C, 75% RH). As the mass of these tablets increased, the diameter and height also increased. In the other cases, the changes were negligible. The hardness against pressure of the tablets decreased in all cases, most strongly for the tablets stored in open containers. The time of disintegration also decreased in all cases, and again the most considerable changes occurred for the tablets stored in open containers. These results are in harmony with the fact that amorphous materials are hygroscopic. In these changes, the presence of Polyplasdone XL 10, as superdisintegrant, plays an important part also. It may be concluded that it is very important to choose the correct conditions for the formulation and storage of amorphous CLP. These conditions include the use of dry air (low RH) and a closed container.

Conclusions

The "in-process" amorphization of CLP as model API was studied, tablets containing amorphous CLP were

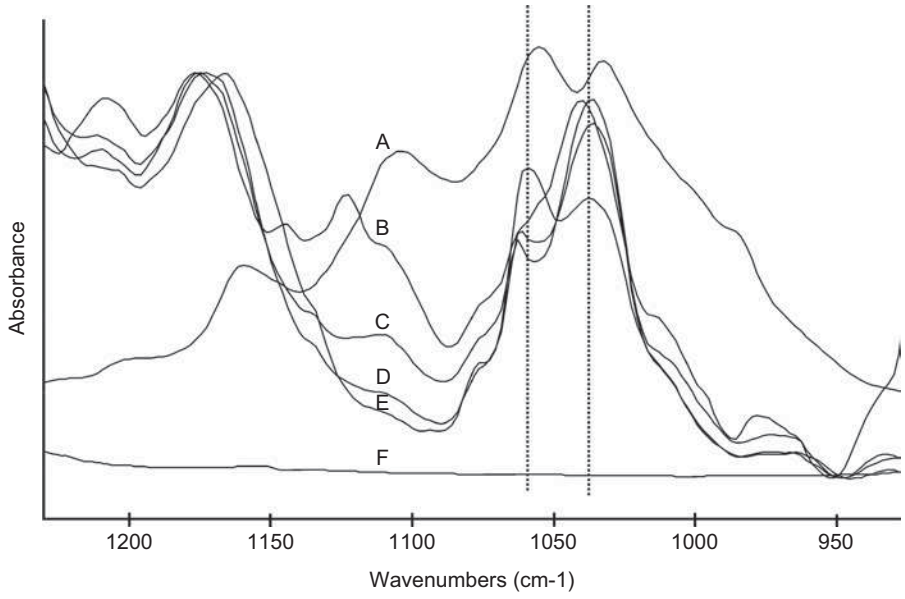

Figure 6. FT-IR analysis of different concentrations of *Sample 2* in solutions containing dichloromethane. **A**: solid *Sample 2*, **B**: 0.1000 g sample in solution of 10 cm^3, **C**: 0.0500 g sample in solution of 10 cm^3, **D**: 0.0250 g sample in solution of 10 cm^3, **E**: 0.0125 g sample in solution of 10 cm^3, **F**: dichloromethane.

produced, and the stability of the product was tested. The results suggested the following amorphization protocol (see Figure 9):

- In the first step, a suitable solvent for the API should be selected. In this step, it is very important that the crystalline API should dissolve completely: any crystals remaining in the system can function as seeds and crystallization can start during evaporation of the solvent.
- In the second step, a suitable crystallization inhibitor should be selected and the optimum amount of this auxiliary agent required to maintain the API in amorphous form should be determined. In this step, several crystallization inhibitors should be screened

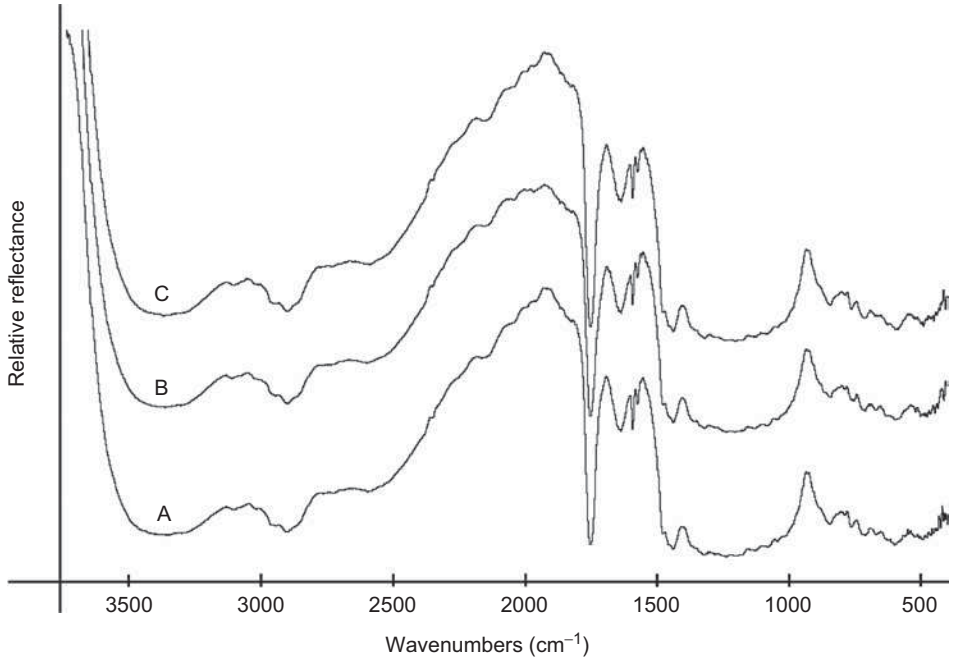

Figure 7. FT-IR investigation of the fresh and stored *Sample 2*. **A**: fresh *Sample 2*, **B**: stored *Sample 2* (40°C, 75% RH, open), **C**: stored *Sample 2* (40°C, 75% RH, closed).

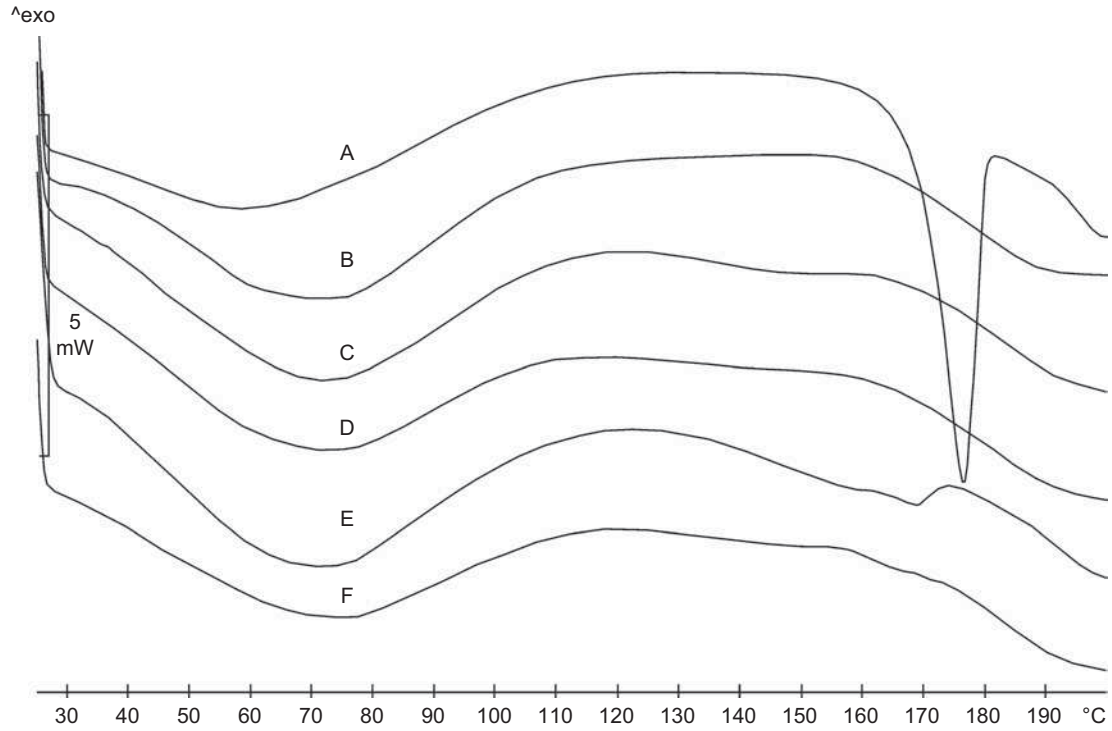

Figure 8. Stability of tablets. **A**: Physical mixture, **B**: fresh product, **C**: stored sample (25°C, 60% RH, open), **D**: stored sample (25°C, 60% RH, closed), **E**: stored sample (40°C, 75% RH, open), **F**: stored sample (40°C, 75% RH, closed).

Table 2. Investigation of tablet parameters.

Tablets		Mass (g)	Diameter (mm)	Height (mm)	Hardness against pressure (*N*)	Time of disintegration (s)
Fresh		0.4060 (SD ± 0.003)	12.10 (SD ± 0.060)	3.50 (SD ± 0.023)	93.4 (SD ± 2.67)	86 (SD ± 27)
Stored 25°C and 60% RH	Open	0.4097 (SD ± 0.004)	12.13 (SD ± 0.050)	3.55 (SD ± 0.032)	70.4 (SD ± 3.63)	30 (SD ± 13)
	Closed	0.4078 (SD ± 0.004)	12.12 (SD ± 0.054)	3.51 (SD ± 0.027)	83.6 (SD ± 3.37)	54 (SD ± 13)
Stored 40°C and 75% RH	Open	0.4106 (SD ± 0.003)	12.24 (SD ± 0.038)	3.69 (SD ± 0.016)	65.8 (SD ± 2.74)	6 (SD ± 4)
	Closed	0.4060 (SD ± 0.003)	12.10 (SD ± 0.009)	3.53 (SD ± 0.021)	84.8 (SD ± 3.79)	79 (SD ± 16)

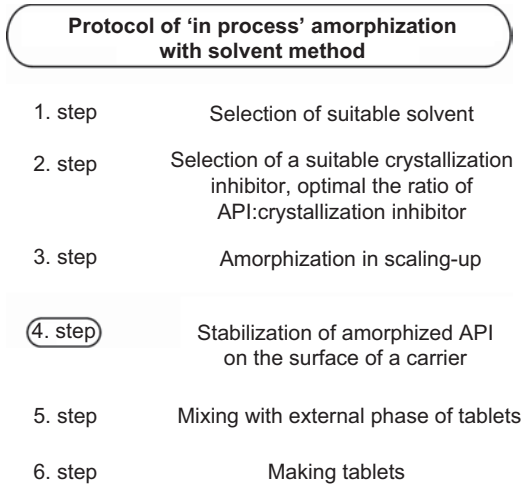

Figure 9. Protocol of 'in-process' amorphization.

and, if possible, the type of interaction between the API and the auxiliary agent should be investigated.

- In the third step, the amorphization process must be scaled up.
- In the fourth step, the amorphized API should be stabilized on the surface of a carrier. This is an important step in this protocol, because the API, the crystallization inhibitor and the carrier act together in this system to result in stable amorphous API during the technological process.
- In the fifth step, the external phase of the tablets should be mixed with the amorphized product. The mixing must be performed very carefully, because mechanical force can induce recrystallization.
- In the sixth step, the tablets should be pressed. Compression can be a further stabilizing step, fixing the amorphous form because of the smaller surface.

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Declaration of interest

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