



Early drug development predictions of glass-forming ability and physical stability of drugs

Denny Mahlin^{a,*}, Christel A.S. Bergström^{a,b}

^a Department of Pharmacy, Uppsala University, Uppsala, Sweden

^b Drug Delivery, Disposition and Dynamics, Monash Institute of Pharmaceutical Sciences, Monash University, Australia

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ABSTRACT

The purpose of this study was to investigate if rapidly measured physical properties can predict glass-forming ability and glass stability of drug compounds. A series of 50 structurally diverse drug molecules were studied with respect to glass-forming ability and, for glass-formers ($n = 24$), the physical stability upon 1 month of storage was determined. Spray-drying and melt-cooling were used to produce the amorphous material and the solid state was analysed by Differential Scanning Calorimetry (DSC) and Powder X-ray Diffraction. Thermal properties and molecular weight (Mw) were used to develop predictive models of (i) glass-forming ability and (ii) physical stability. In total, the glass-forming ability was correctly predicted for 90% of the drugs from their Mw alone. As a rule of thumb, drugs with Mw greater than 300 g/mole are expected to be transformed to its amorphous state by using standard process technology. Glass transition temperature and Mw predicted the physical stability upon storage correctly for 78% of the glass-forming compounds. A strong sigmoidal relationship (R^2 of 0.96) was identified between crystallization temperature and stability. These findings have the potential to rationalize decisions schemes for utilizing and developing amorphous formulations, through early predictions of glass-forming ability from Mw and physical stability from simple DSC characterization.

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1. Introduction

When developing a dosage form for an active compound with poor solubility, preformulation data give rather unspecific information on what formulation strategies to apply (Branchu et al., 2007; Kawabata et al., 2011). A higher degree of prediction and precision in decision making would enable more efficient drug product development and provide an early stage insight into the potential of solubility limited drug compounds to be processed into functional and stable dosage forms. In this context, it is necessary to develop methods that can predict the solid state behaviour of drug compounds during processing and manufacturing. Solid state alterations, in particular amorphization, often have significant influence on the performance of a substance, impacting for instance mechanical properties (Ziffels and Steckel, 2010), dissolution (Lindfors et al., 2006; Murdande et al., 2010) and bioavailability (Hancock and Parks, 2000). Amorphization is hence a strategy with high potential to increase bioavailability of compounds for which poor solubility is limiting intestinal absorption. However, as the inherent instability of the amorphous state limits

production, handling and use of products based on amorphous compounds, research efforts are currently directed towards methods that stabilize the amorphous phase (Kearns et al., 2008; Laitinen et al., in press). Fundamental aspects governing the physical stability, i.e. the resistance of an amorphous compound to be transformed into its crystalline state, has lately been in focus with the purpose to obtain an increased understanding of the dynamics (Aso et al., 2001; Bhattacharya and Suryanarayanan, 2009; Singh and de Pablo, 2011; Stukalin et al., 2009) and nucleation processes (Marsac et al., 2006; Vyazovkin and Dranca, 2007).

Thermodynamically the physical stability is governed by the difference in Gibbs free energy between the amorphous and the crystalline states. Both nucleation rate and crystal growth is however also affected by the dynamics, i.e. the molecular mobility, of the amorphous phase. The glass transition temperature (T_g) has therefore been used as a reference temperature when determining glass-formation temperatures (Corrigan et al., 2004; Yamaguchi et al., 1992) and storage temperatures (Hancock et al., 1995; Schoug et al., 2009). However, the predictive capacity of T_g for physical stability has been shown to be poor, which is manifested, by for instance, the observation that compounds with similar T_g may have different amorphous stability (Marsac et al., 2006), and that alterations in amorphous stability attained by variations in production settings not always are reflected in observable changes of T_g (Yamaguchi et al., 1992; Zhang et al., 2009).

* Corresponding author. Address: Department of Pharmacy, Uppsala University, Uppsala Biomedical Center, P.O. Box 580, SE-751 23 Uppsala, Sweden. Tel.: +46 18 4714662; fax: +46 18 4714223.

E-mail address: denny.mahlin@farmaci.uu.se (D. Mahlin).

Some recent publications have described the use of statistical methodology to find other physicochemical properties that correlate with glass-forming ability and glass stability. In these, the interest has been to predict and understand which compounds can be made stable amorphous based on the thermodynamic and kinetic properties of the compounds. Greaser et al. made univariate correlation analysis of kinetic and thermodynamic parameters to assess storage stability of nine drug compounds and found configurational entropy to be the parameter that best described the stability (Graeser et al., 2009). In another study, logistic regression analysis was used to find that T_g and molecular volume combined predict glass-forming ability for a number of compounds when exposed to mechanical treatment (milling) (Lin et al., 2009). Taylor and co-workers have analysed a larger dataset of compounds ($n = 51$) by principal component analysis (PCA) and found that molecular properties (number of rotational bonds and molecular weight) are important, but also that thermal properties (heat of fusion, entropy of fusion, the free energy difference between the crystalline and amorphous states and melting temperature) need to be included to separate glass-formers from poor glass-forming compounds (Baird et al., 2010). The same factors were found to be important for discriminating fast, intermediate and slow crystallizers in a follow up study on physical stability of amorphous drugs (Van Eerdenbrugh et al., 2010). Although these attempts have identified some properties that likely will influence the stability of the amorphous material, no conclusions have been reached on the understanding of the fundamental properties governing amorphous phase formation and stability of drug like compounds (Bhugra and Pikal, 2008).

Recently we have shown how statistical modelling by partial least squares projection to latent structures discriminant analysis (PLS-DA) can be used to predict glass-forming ability of compounds from their molecular structure (Mahlin et al., 2011). The establishment of a model that used molecular descriptors reflecting size, branching, distribution of electronegative atoms, symmetry and number of benzene rings correctly predicted 75% of the compounds in an external test set. In the present work, we continued to explore the inherent ability of pure drugs to form an amorphous state in settings comparable to standard production conditions. A series of 50 structurally diverse drugs was investigated upon processing by spray-drying and melt-cooling. For the compounds thereby showing good glass-forming ability we further studied the inherent ability to remain in the amorphous state upon storage. This resulted in two datasets; a dataset for the ability to form the glass, in which the compounds were sorted as (i) glass-former or (ii) nonglass-former, and a dataset for the stability of the formed material, in which the compounds ($n = 24$) were classed as (iii) stable glass or (iv) non-stable glass. The datasets were used together with experimentally measured physical properties to develop models predicting glass-forming ability and glass stability, applicable as preformulation tools in early drug development.

2. Methods

2.1. Materials

In this study, a dataset previously investigated for glass-forming ability (Mahlin et al., 2011), and for which most of the compounds display solid-state limited aqueous solubility, was extended with a diverse set of molecules to allow general conclusions to be drawn applicable to the drug-like space of oral drugs. In total 50 compounds were included in the final dataset subjected to analysis of properties of importance for glass-forming ability and glass stability (Table 1). All of the compounds studied were used in their free

form, i.e. no salts of compounds were included. Differential Scanning Calorimetry (DSC) verified that the starting material was crystalline and none of the compounds showed any traces of solvates. Bicalutamide, felodipine and linaprazan were received as a kind gift from AstraZeneca (Mölnådal, Sweden) and acitretin was purchased from Ontario Chemicals (Canada). All the other drugs were obtained from Sigma–Aldrich Chemie GmbH (Germany). The specified purity of the drugs used was >98%, except for griseofulvin (>96%). Ethanol (Alita Corporation, Finland) and acetone (VWR International S.A.S., France) were used as solvents in the spray-drying feed solution.

2.2. Production and identification of the amorphous form

Two different methods, spray-drying and melt-cooling, were used to test the susceptibility of the compounds to be transformed into the amorphous form. Only the compounds for which both these methods resulted in the same outcome, i.e. formation of either a crystalline or an amorphous solid, were included in the dataset that was utilized for statistical modelling. The dual production procedure was applied for two reasons. Firstly, the idea was to identify the inherent glass-forming ability of the drug compounds rather than the process dependent glass-forming properties. Secondly, we wanted to minimize the risk of false classification that may be caused by hidden processes that affect the outcome, such as chemical degradation upon heating. Melt-cooling was done in DSC using unprocessed substance and spray-drying by using solutions of the compounds as described in detail previously (Mahlin et al., 2011). Briefly, the solubility of each compound in a solvent mixture of ethanol and acetone (90:10 w/w) was determined by preparing a dispersion of the drug in the solvent mixture, which was subsequently stepwise diluted and sonicated until complete dissolution was observed. Solutions of the compounds at a concentration corresponding to 75% of the solubility were spray-dried in a Büchi B-290-Mini Spray Dryer with an inert loop (Büchi Laboratories, Switzerland) using a standardised procedure with the following settings: inlet temperature 50 °C, pump rate of spray solution 4 ml/min, and aspirator rate 75% of the maximum flow. The produced material was dried over vacuum at room temperature (22 °C) for 1 h prior to solid state analysis.

The solid state of the spray-dried material was analysed by DSC (DSC6200, Seiko, Japan). The temperature and heat flow was calibrated using indium. Approximately 2 mg of each compound were weighed into aluminium pans (TA Instrument standard pan, Waters, UK) with perforated lids and analysed using a heating rate of 20 °C/min enabling full separation of crystallization and melting peaks. The analysis was run from 20 °C to a temperature above the melting point of the compound (T_m) while being purged with nitrogen gas (80 ml/min). No signs of residual solvents desorbing during heating was observed in the DSC signal. The presence of amorphous phase in the samples was judged from the occurrence of glass transition and exothermic crystallization peaks in the heat flow signal upon heating, alternatively a complete absence of crystallization and melting peaks. The glass transition was determined from the mid-point of the step change in heat flow and the amorphous content of the spray-dried compounds was estimated from:

$$\% \text{Amorphous} = \frac{\Delta H_{cr}}{\Delta H} 100 \quad (1)$$

where ΔH_{cr} is the enthalpy of crystallization and calculated from area under the crystallization peak in the thermogram, and ΔH is the difference in enthalpy between the amorphous and crystalline state at the crystallization temperature (T_{cr}), and given by

$$\Delta H = \Delta H_m - \int_T^{T_m} \Delta C_p dT \quad (2)$$

where ΔH_m is the melting enthalpy, T_m the melting temperature and

$$\Delta C_p = C_p^{am} - C_p^{cr} \quad (3)$$

where C_p^{am} and C_p^{cr} are the heat capacities of the amorphous and crystalline state, respectively. As an approximation, ΔC_p can be assumed to be constant and calculated according to Thompson and Spaepen (1979):

$$\Delta C_p = \frac{\Delta H_m}{T_m} \quad (4)$$

where ΔH_m and T_m is obtained from the DSC data.

The solid state of the spray-dried material was further verified by X-ray Powder Diffraction analysis. Diffraction patterns were obtained by using a Kratzky camera with a linear position-sensitive wide angle detector (HECUS M. BRAUN X-ray Systems, Graz, Austria) detecting diffracted radiation in a 2θ interval from 17° to 25° (given by the limits of the detector) in steps of 0.01° . The radiation was generated by an Cu $K\alpha$ X-ray generator (Philips, PW 1830/40) working at 40 V and 50 A. The temperature was controlled to 25°C by a Peltier element. Each sample was run for 15 min in vacuum. When the X-ray analysis showed a diffuse scattering pattern the sample was considered to be predominantly amorphous, while samples generating diffraction patterns with distinctive peaks were considered to contain crystalline phase.

Table 1
Physicochemical and glass stability characteristics of the compounds studied.

Compounds	T_g ($^\circ\text{C}$)	T_m ($^\circ\text{C}$)	T_{cr} ($^\circ\text{C}$)	ΔH_m (J/g)	ΔG_{cr} (J/g)	ΔS_m (J/K/g)	Mw (g/mol)	Glass-former	Stable on storage?
Acemetacin	37	148	79	135	40	0.32	415.8	Yes	No
Acetohexamide	26	190	65	206	75	0.44	324.4	Yes	No
Albendazole ^a	60	202	56	202	77	0.43	265.3	Yes	No
Bezafibrate	73 ^a	184	61	176	62	0.39	361.8	Yes	No
Carbamazepine	61 ^b	190	77	111	40	0.24	236.3	Yes	No
Felodipine	41	142	86	85	25	0.21	384.3	Yes	No
Griseofulvin	88	216	80	123	49	0.25	352.8	Yes	No
Nifedipine	45 ^b	171	73	134	45	0.30	346.3	Yes	No
Testosterone	42	153	51	109	34	0.26	288.4	Yes	No
Bicalutamide	50	192	97	123	45	0.26	430.4	Yes	Yes
D-lactose	105	195	140	100	37	0.21	342.3	Yes	Yes
Famotidine	50	167	104	185	61	0.42	337.4	Yes	Yes
Glafenine	63	164	120	87	28	0.20	372.8	Yes	Yes
Glibenclamide	62	172	–	116	39	0.26	494.0	Yes	Yes
Hydrochlorothiazide	110	263	139	144	65	0.27	297.7	Yes	Yes
Hydrocortisone	86	211	123	118	46	0.24	362.4	Yes	Yes
Hydroflumethiazide	100	269	142	122	56	0.23	331.3	Yes	Yes
Indometacin	44	159	104	122	39	0.28	357.8	Yes	Yes
Linaprazan	100	246	147	148	64	0.29	366.5	Yes	Yes
Metolazone	109	266	–	109	49	0.20	365.8	Yes	Yes
Omeprazole	51	155	90	145	45	0.34	345.4	Yes	Yes
Pimozide	57 ^b	216	106	100	40	0.20	461.5	Yes	Yes
Spirolactone	91	213	127	60	23	0.12	416.6	Yes	Yes
Warfarine	68	162	–	195	63	0.45	308.3	Yes	Yes
Bucetin	–3 ^a	158	–	159	50	0.37	223.3	No	–
Bufexamac	2 ^a	167	–	93	31	0.21	223.3	No	–
Caffeine	72 ^b	236	–	101	42	0.20	194.2	No	–
Chlorzoxazone	38 ^b	190	–	161	58	0.35	169.6	No	–
Diflunisal	43 ^a	213	–	175	69	0.36	250.2	No	–
D-mannitol	13 ^c	165	–	374	122	0.85	182.2	No	–
Fenbufen	28 ^a	186	–	202	72	0.44	254.3	No	–
Flumequine	78 ^a	258	–	147	65	0.28	261.2	No	–
Hymecromone	–9 ^a	181	–	202	71	0.44	176.2	No	–
Indoprofen	50 ^b	210	–	143	56	0.30	281.3	No	–
Mefenamic acid	51 ^a	231	–	214	89	0.42	241.3	No	–
Naproxen	–3 ^a	153	–	157	49	0.37	230.3	No	–
Pindolol	14 ^a	169	–	263	87	0.60	248.3	No	–
Primidone	72 ^a	283	–	211	99	0.38	218.3	No	–
Probenecid	50 ^a	199	–	153	57	0.32	285.4	No	–
Pyrazinecarboxamide	–28 ^a	188	–	209	75	0.45	123.1	No	–
Saccharin	20 ^a	226	–	189	77	0.38	183.2	No	–
Salicylic acid	–38 ^a	158	–	205	65	0.48	138.1	No	–
Spiperone	106 ^a	207	–	162	62	0.34	395.5	No	–
Sulfamethoxazole	16 ^a	169	–	156	52	0.35	253.3	No	–
Sulfanilamide	–21 ^a	164	–	175	57	0.40	172.2	No	–
Theophylline	94 ^b	272	–	185	85	0.34	180.2	No	–
Tolfenamic acid	63 ^b	212	–	144	56	0.30	261.7	No	–
Trimethoprim	51 ^a	197	–	167	62	0.36	290.3	No	–
Tyramine	–37 ^a	161	–	262	84	0.60	137.2	No	–
Zoxazolamine	11	183	–	191	67	0.42	168.6	No	–

Glass transition temperature (T_g), onset temperature of melting (T_m), onset temperature of crystallization (T_{cr}), enthalpy of melting (ΔH_m), Gibbs' free energy of crystallization (ΔG_{cr}), entropy of melting (ΔS_m) and molecular weight (Mw). Compounds showing detectable amounts of amorphous phase directly after spray-drying and melt-cooling were classified as glass-formers.

^a Calculated according to Baird et al. (2010) (24).

^b Value obtained from Baird et al. (2010) (24).

^c Value from SciFinder (ACS).

The ability of the compounds to become amorphous when cooled from the pure liquid state was investigated by cooling melts of the drugs in the DSC. The experimental conditions were the same as for the analysis of spray-dried material, except that approximately 2 mg of unprocessed substance was weighed into the aluminium pans. The samples were analysed by performing two heating/cooling cycles, the first for melt-cooling and the second for analysis. During the first cycle the samples were heated from room temperature to approximately 10 °C above their T_m at a heating rate of 20 °C/min and immediately cooled at a rate of 40 °C/min. To analyse the presence of an amorphous phase after the first cycle, the second DSC cycle was performed in the same way as the DSC run to determine the amorphous content of the spray-dried material. No thermal events other than the expected glass transitions, crystallizations and melting, were observed in the DSC signal upon heating of the material.

2.3. Dry stability of amorphous material

The spray-dried material of the pure drug compound was put on short term storage to provide an indication of the dry stability of the glass-formers when kept in the glassy state, below their T_g . Therefore, all compounds being partially or completely amorphous after spray-drying were stored for 1 month in glass vials over silica gel in an evacuated desiccator at room temperature (22 °C). The solid state of each compound was then analysed again by DSC applying the same DSC protocol as used immediately after production. The fraction of the amorphous phase that had been transformed into a crystalline state upon 1 month of storage (α) was calculated by

$$\alpha = 1 - \frac{\Delta H_{cr}^{stored}}{\Delta H_{cr}} \quad (5)$$

where ΔH_{cr}^{stored} is the heat of crystallization of the solid after storage and ΔH_{cr} the same as above, i.e. heat of crystallization determined immediately after spray-drying.

2.4. Analyses of glass-forming ability and dry stability – relation to physical properties

The glass-forming ability and dry stability were analysed for their dependence of the following measured physical properties which were obtained from DSC analysis of the unprocessed crystalline material: T_m (onset of melting peak), ΔH_m (melt enthalpy from melting peak area), ΔS_m (entropy of melting) and ΔG_{cr} (Gibbs free energy of crystallization at storage temperature), and analysis of amorphous material obtained after spray-drying: T_g (the midpoint of the glass transition temperature) and T_{cr} (onset of crystallization temperature upon heating at 20 °C/min). In addition, Mw, which previously has been identified as an important molecular property for glass-forming ability (Baird et al., 2010) and the following adjusted properties were included: reduced T_g ($T_{g,red}$ which is equal to the ratio T_g/T_m), $T_m - T_g$, $(T_{cr} - T_g)/(T_m - T_g)$, $\Delta G_{cr} \times T_{g,red}$, $\Delta G_{cr}/T_{g,red}$, $\Delta G_{cr} \times T_g$, $\Delta G_{cr}/T_g$, $\Delta G_{cr} \times Mw$, $\Delta G_{cr}/Mw$, $T_m \times Mw$, T_m/Mw , $T_g \times Mw$, T_g/Mw , $T_{g,red} \times Mw$, $T_{g,red}/Mw$, $T_{cr} \times T_g$, T_{cr}/T_g , $T_{cr} \times Mw$, T_{cr}/Mw , $\Delta G_{cr} \times T_{cr}$ and $\Delta G_{cr}/T_{cr}$. These adjusted parameters were introduced to make possible the finding of relations between parameters that are non-linearly interdependent. An estimated value of T_g was calculated for compounds for which T_g could not be determined from the thermal analysis, using a procedure described by Baird et al. (2010). In short, the $T_{g,red}$ of the compounds for which T_g had been experimentally determined was plotted as a function of Mw. A straight line was fitted to the plot and thereby a theoretical T_g could be calculated from the obtained straight line equation.

All the above described properties were included as variables and subjected to PLS-DA as implemented in Simca v.11 (Umetrics, Sweden). The following procedure was used to develop the classification model of glass-forming ability: The computational model for glass-forming ability ($n = 50$) was designed to differentiate between glass-formers, i.e. compounds able to form an amorphous state from both spray-drying and melt-cooling (assigned value 1), and non-glass-formers, i.e. compounds remaining crystalline irrespective of production technology used (assigned value -1). This classification neither took into account how much of the material that had become amorphous upon processing nor whether the amorphous material was stable over time; only the ability to exist in the amorphous state after being subjected to the two material processing techniques was modelled. It should here be noted that the melt-quenching and spray-drying are two fundamentally different routes for solid formation, the former a transformation from the melted state and the latter from a solution. This should certify that we are studying the inherent glass-forming propensity of the drugs. The dataset was divided into training (2/3) and test (1/3) sets to allow assessment of general applicability of the models developed. Standard settings in Simca, including seven cross validation groups, were used.

The model for glass stability was devised to separate stable glasses, defined as compounds which had retained more than 50% of the amorphous content after 1 month of storage (assigned the value 1), from non-stable glasses defined as compounds that lost more than 50% of the amorphous content during this time period (assigned value -1). Albendazole was excluded from the stability modelling due to its high crystalline content after production (82%) which possibly could obscure a correct analysis of the stability of the amorphous phase and hence increasing the risk of false classification. Due to the small number of compounds ($n = 23$) and that the number of compounds in the stable group ($n = 15$) was large compared to the unstable group ($n = 8$), all the compounds were included in the model development. To give the two groups equal weight, the unstable group was duplicated in the input matrix used for PLS-DA, resulting in that information from the same compound was repeated eight rows down in the matrix. This approach has been identified as suitable when modelling significantly different group sizes. In the model development of dry stability the number of cross validation groups was set to eight in order to simultaneously leave both duplicates out in the cross-validation of the model.

In the model development for both glass-forming ability and stability, the data were mean centered and scaled to unit variance, and variables that were skewed were excluded from the model development to not distort the models. The models were developed using the following schedule to allow as much information to be extracted as possible: (i) only thermodynamic properties (T_m , ΔH_m , ΔS_m , ΔG_{cr}) were used as input variables ($n = 4$), (ii) to matrix i, T_g related properties were added ($n = 11$) and (iii) to matrix ii, Mw related properties were added ($n = 20$). In the modelling of glass stability matrix iv was created by adding T_{cr} related properties were to matrix iii ($n = 29$). From each starting point (i-iv) a variable selection was performed in which input information that was not directly related to the response (i.e., noise) was removed, and thereby the predictivity and robustness of the model was increased. The accuracy of the statistically significant PLS-DA models was judged by how well the two classes of the training sets were separated from each other. In addition, for glass-forming ability, once the selection of physical properties had been finalized the resulting models were validated with the test set.

To evaluate the models for glass stability, the fraction of the amorphous phase that had been transformed into a crystalline state upon 1 month of storage (α) was plotted against T_g , Mw, T_{cr}

and the prediction values obtained from the PLS-DA model based on T_g and Mw. A sigmoidal relationship

$$\alpha = 1 - \frac{1}{(1 + e^{(T_0 - T_{cr})k})} \quad (6)$$

was fitted to the data points in the plots by adjustment of the shape factors T_0 and k .

3. Results and discussion

3.1. Preparation of amorphous materials – glass-forming ability

The results from the classification of glass-forming ability of the 50 compounds are presented in Table 1. For all compounds there was an agreement between DSC and X-ray data, as a clear crystallization peak visible in the thermogram upon heating in all cases coincided with a diffuse background scattering without diffraction peaks in X-ray. In the case of glibenclamide, metolazone and warfarine, the absence of both a crystallization peak and a melting peak in the DSC thermogram was taken as the sample being amorphous and stable upon heating. The X-ray analysis of these compounds confirmed they being predominantly amorphous state. Albendazole and Nifedipine showed small crystallization peaks and estimations based on the DSC-data showed that were just partially amorphous (approximately 18% and 67%, respectively). Of the 50 compounds investigated, 26 were detected to be crystalline (no amorphous phase detected) after both melt-cooling and spray-drying whereas 24 showed partly or complete transformation to the amorphous form. Hence, the latter 24 were classified as glass-formers (see Table 1).

3.2. Stability of dry amorphous material

After storage for 1 month, DSC-analysis showed that 15 of the glass-formers had preserved more than 50% of its amorphous content (see Table 1). For 13 of these, the fraction crystallized was <5% which is within the uncertainty of the crystallinity determination by this method. Bicalutamide and omeprazole lost approximately 11% and 36% of their amorphous content, respectively. For the compounds classified as unstable, no amorphous phase could be detected by DSC after storage, except for griseofulvin, felodipine and acemetacin, which according to our calculations had a crystallinity of 95%, 79% and 56%, respectively, after storage.

3.3. Physical properties of importance for glass-forming ability of drugs

In order to find out if thermodynamic properties could be used for predictions of glass-forming ability a PLS-DA was performed based on the thermodynamic quantities T_m , ΔH_m , ΔS_m and ΔG_{cr} . These are easily measured by using a crystalline sample of a compound using standard DSC equipment. However, the PLS-DA modelling attempts resulted in non-significant models (data not shown). In the next step, we therefore also included T_g -related parameters, which are assumed to represent properties related to the molecular mobility of the amorphous state. Interestingly, the most predictive model, shown in Fig. 1, did not include any parameter representing an absolute temperature parameter (T_m or T_g), as could be expected since the quality of the amorphous product formed often are related to difference between formation temperature and T_g (Yamaguchi et al., 1992). Instead it was the balance between thermodynamic and kinetic properties, i.e. the adjusted parameters involving both T_m and T_g , that carried most information. In this case, the predictivity was 81% for the test set (Fig. 1A). The model was based on $T_{g,red}$, $T_m - T_g$, ΔS_m , $\Delta G_{cr} \times T_{g,red}$, ΔH_m , $\Delta G_{cr}/T_{g,red}$ and $\Delta G_{cr}/T_{g,red}$ and hence, the analysis showed that

the T_g -related properties indeed carry information of importance for the prediction of glass-forming ability.

In a general context, larger molecules are commonly less prone to crystallize from a liquid state (Baird et al., 2010). Therefore, we wanted to evaluate the effect of Mw on the predictions and hence, a new model was built including all former parameters, together with Mw-related properties. In this analysis, only the adjusted parameter $T_{g,red} \times Mw$ remained after model refinement and this property predicted 91% and 94% correctly of the training and test sets, respectively (Fig. 1B). We also found that equal predictivity was obtained from Mw alone (accuracy of training and test sets of 88% and 94%, respectively, Fig. 1C). The results obtained herein, based on a large and structurally diverse drug-like dataset, strengthen previous findings of the importance of molecular size and T_g as predictors of glass-forming ability (Lin et al., 2009). In the scientific discussion, it is often referred to Kauzmann (1948) and Turnbull (1969) who suggested that compounds with a $T_{g,red}$ higher than 2/3 are good glass-formers. The theoretical rationale for this effect is that compounds with smaller super-cooled liquid regime (i.e. high $T_{g,red}$) have a lower probability for nucleation when cooled below its melting temperature due to less time spent in that critical region. This has been confirmed in a study on a homologous series of cyclic stilbenes (Ping et al., 2011), but in the same publication it was argued not to be true when looking at more diverse chemical structures. Recently it was shown by Baird et al., that for a set of drug compounds the $T_{g,red}$ is not useful for predicting glass-forming ability (Baird et al., 2010). This is partially in line with our observation that Mw is a good predictor by itself, and that the $T_{g,red}$ contributes with minor information. This was further underlined by that $T_{g,red}$ alone was a poor predictor (data not shown). We therefore propose that for compounds with a molecular weight range corresponding to common poorly soluble drugs, properties relating to molecular size is the dominating factor determining glass-forming ability, whereas for limited series of compounds with similar molecular weight, the $T_{g,red}$ may be more useful for predictions. Some publications highlight the role of the configurational entropy difference between the amorphous and crystalline state, and that compounds with higher Mw have more complex molecular structure and hence, are less likely to exist in an ordered crystalline state (Bhugra and Pikal, 2008; Graesser et al., 2009; Zhou et al., 2002). Therefore, there seems to be a rational behind using the Mw as an easily obtained surrogate for description of configurational entropy, although the latter property also is dependent on other structural features, e.g. number of rotatable bonds. Further, it has been suggested that the complexity associated with larger molecules means that it has to probe a larger number of possible conformations and configurations to find an ordered (crystalline) packing structure during solidification (Bhugra and Pikal, 2008). It is appealing to imagine the tendency of becoming either amorphous or crystalline as being dependent on the molecular process of probing the various possible conformations and configurations (related the configurational space, and hence to the Mw of the compound) and the time available to find a configuration that will produce an ordered crystal unit during solidification (related to the $T_{g,red}$ at constant cooling conditions).

In the present study, the dominating factor for glass-formation seems to be Mw. In Fig. 2 the relation between Mw and glass-forming ability is visualized. From our analysis, based on a large structurally diverse dataset we suggest that compounds with Mw above 300 g/mole are likely to be transformed to the corresponding glass using standard production/amorphization technologies, whereas compounds with Mw below this value will be difficult to produce amorphous. It should be kept in mind that we base this conclusion on compounds having a melting point higher than 140 °C. However, the general applicability of this rule-of-thumb was confirmed by applying the analysis on the 51 compounds studied by Baird

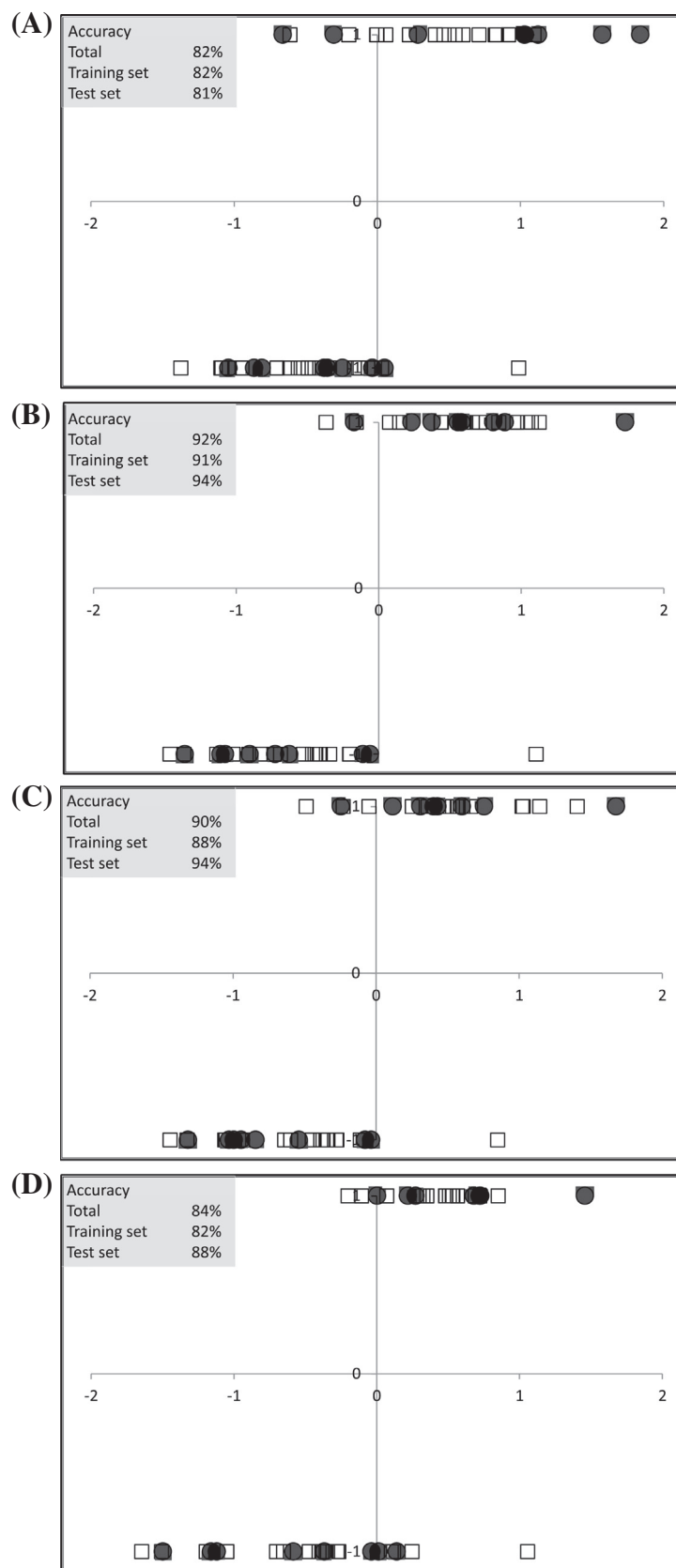


Fig. 1. Accuracy of the prediction of the glass-forming ability for the training set and the test set used. Training set used for model development is shown as open squares, test set as filled circles. Glass-formers were given the value 1 and nonglass-formers (i.e. compounds remaining crystalline after being processed by both spray-drying and melt-cooling) were given the value -1 . Observed glass forming ability is shown on the y-axis, predicted on the x-axis. (A) Model based on thermodynamic and kinetic glass properties. Remaining after model optimization were $T_{g,red}$, ΔH_m , ΔS_m , $T_m - T_g$, $(\Delta G_{cr}/T_{g,red})$, $(\Delta G_{cr}/T_g)$, $(\Delta G_{cr} \times T_{g,red})$. (B) Model obtained after adding Mw related properties to the variable selection. $T_{g,red} \times Mw$ remained after variable selection. (C) Classification obtained from Mw alone. (D) Classification based on $T_{g,red}$ alone.

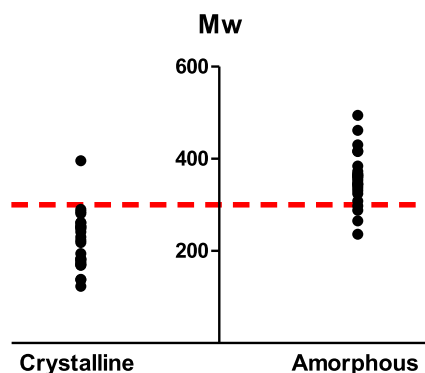


Fig. 2. Single correlation between glass-forming ability and molecular weight. Based on the results from the 50 compounds studied herein a cut-off for Mw of 300 is suggested for rapid prediction of glass-forming ability, with compounds having Mw > 300 mg/mole likely to be good glass-formers.

et al. (2010). For this dataset, 84% of the compounds were correctly sorted with regard to their glass-forming ability when using Mw of 300 g/mole as the cut-off value.

3.4. Physical properties of importance for dry stability of amorphous drugs

In the same way as for glass-forming ability, the glass stability was analysed step-wise. The thermodynamic properties did, again, not result in a significant model for dry stability. The variable selection after including the T_g -related properties to the model development resulted in that T_g was found to be the single most important property, and did by itself predict 65% of the compounds accurately (Fig. 3A). After inclusion of Mw the final properties remaining after the variable selection were T_g and Mw and the predictivity increased to 78% (Fig. 3B). It should be noted that all T_g -values except one (bezafibrate) used in stability modelling have been experimentally determined.

Since no test set was available for validation, the stability model developed was evaluated using the calculated fraction of the amorphous phase transformed during storage (α). A plot of α as a function of the prediction values generated by the model is displayed in Fig. 4. This shows the model is not only able to separate the two classes stable and non-stable with 78% certainty, but also able to assign the lowest values (<-0.5) for all the compounds that was fully crystallized upon storage, and highest values (>0) for all the compound that did not crystallize during storage (the only exception being griseofulvin having high prediction value but low stability). There seem to be a sigmoidal relation between the predicted values and α which further support the validity of the model. The rationale for why a model based on the parameters T_g and Mw is able to predict glass stability can be deduced in a similar way as for glass-forming ability, i.e. it is the balance between the molecular mobility (the rate of molecular motion) and the configurational space (how many configurations that can be probed) that governs crystallization tendency of a compound. It has been shown that molecular mobility determines the rate of crystallization of an amorphous phase when analysing the temperature dependency of single amorphous compounds (Aso et al., 2001; Bhattacharya and Suryanarayanan, 2009; Bhugra et al., 2008). However, when it comes to comparing crystallization tendencies for a number of structurally diverse compounds other factors has to be considered to predict physical stability (Van Eerdenbrugh et al., 2010) and one factor identified to be important is the configurational entropy (Graeser et al., 2009; Zhou et al., 2002). Based on this we hypothesize that T_g and Mw is describing

molecular mobility and configurational entropy well enough to, when combined, be able to predict glass stability. It is interesting to note that the compound being poorest predicted by the Mw- T_g -storage model, griseofulvin, has been extensively studied as to find out the reason for its sensitivity to production conditions, since its stability is higher when amorphized by melt-cooling as compared to milling (34–36).

3.5. Crystallization temperature

A glass heated above its T_g may crystallize before it reach the thermodynamic melting temperature. The onset of this crystallization is dependent on the nucleation tendency and crystal growth rate of the heated amorphous system (Bhugra and Pikal, 2008; Hancock and Zograf, 1997). At a well-defined heating rate and sample size, the onset temperature of crystallization (T_{cr}) can be regarded as an indicator of the crystallization tendency of the amorphous compound. However, no evaluation of the capacity of T_{cr} to predict storage stability for a number of relatively diverse drug compounds has so far been published. Since we in this study had information on physical stability of the amorphous phase upon storage below T_g we had an opportunity to study its relation to T_{cr} . Hence, T_{cr} was included as an input parameter and evaluated by the PLS-DA modelling. In the refined model T_{cr} remained as the only parameter, on its own giving the best predictivity, with 95% accurate classification of the compounds (Fig. 3C). To further evaluate this correlation a plot of α as a function of the T_{cr} was done. As for the stability prediction model a strong sigmoidal relationship (R^2 of 0.96 upon fitting to Eq. (6)) was obtained (see Fig. 4). No clear outliers from this relation were found, indicative of that T_{cr} is able to capture the important factors that govern the physical stability of amorphous compounds upon storage below T_g .

Although the relation between molecular mobility and crystallization of amorphous compounds below and above T_g has been studied previously (Bhugra et al., 2008; Caron et al., 2010), such a clear and simple correlation between T_{cr} and storage stability as the one observed here has, to the best of our knowledge, not been reported. T_{cr} has shown to be sensitive to the condition of an amorphous material in terms of physical aging (Surana et al., 2004) and pre-nucleation (Trasi et al., 2010; Wu and Yu, 2006) which in turn is dependent on the production setting and thermal history of the amorphous phase. Hence, it seems logical that T_{cr} better describes the stability than Mw and T_g , since the latter can be regarded more as intrinsic material properties. Therefore, it is very likely that the T_{cr} better correlates to storage stability of amorphous materials produced by different technologies and at different conditions. However, further studies are needed to confirm this assumption. From a prediction perspective, the 78% accuracy obtained using T_g and Mw justify the usage of these properties to predict the inherent glass stability of compounds in the early part of the drug development process, since T_g may be estimated from calculations (Baird et al., 2010) or simulations (Xiang and Anderson, 2013) *in silico*. However, T_{cr} may more accurately foresee stability later during the drug development process, in particular during stages when decisions are to be made with regard to preferred production technology for the amorphization.

From the plot in Fig. 4, it is apparent that a compound with a T_{cr} higher than 100 °C is stable upon 1 month of storage at 22 °C. This relation can also be expressed as that an amorphous compound has to be stored at no less than 80 °C below its T_{cr} in order to be stable for 1 month, and is valid for T_{cr} -values determined at a heating rate of 20 °C/min. However, the validity for other storage temperatures, relative humidities and formulations compositions must be further evaluated.

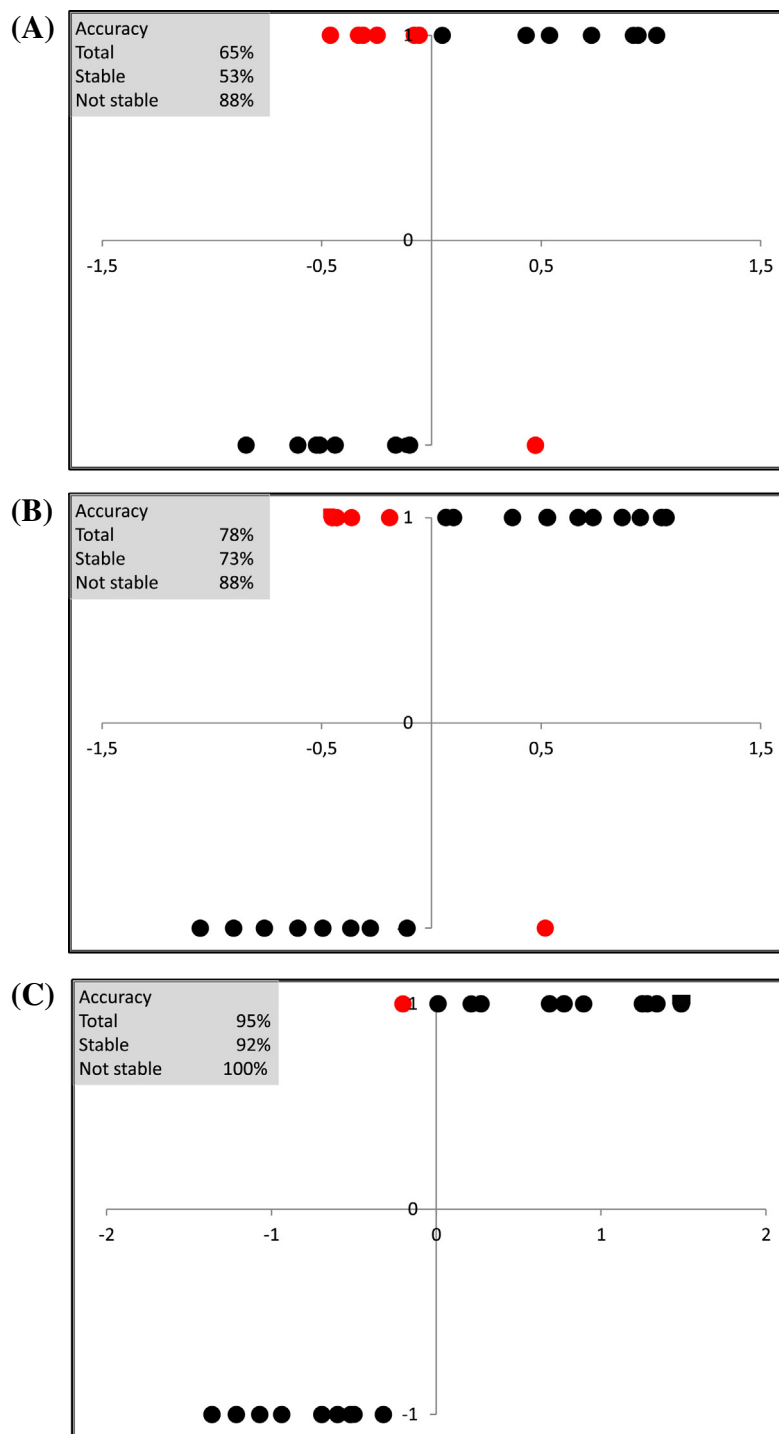


Fig. 3. Accuracy of the prediction of the glass stability for the training set used. Correctly predicted compounds are shown as black circles, falsely predicted in red. Compounds being stable amorphous were given the value 1, whereas unstable compounds were given the value -1 , and all experimental data obtained in this study was used in the analysis of properties of importance for amorphous stability. Observed glass stability is shown on the y -axis, predicted on the x -axis. (A) Model based on thermodynamic and kinetic glass properties. T_g alone remained after variable selection. (B) Model obtained after adding Mw related properties to the variable selection. Final model obtained was based on T_g and Mw. (C) Model obtained after adding T_{cr} related properties to the variable selection. The final model obtained was based on T_{cr} only.

4. Conclusion

In this work we have studied a large series of structurally diverse compounds and from thermal analysis and multivariate data analysis identified important properties to forecast the inherent drug properties glass-forming ability and dry stability of the

produced amorphous material. The glass-forming ability was shown to be well predicted from Mw alone. The results suggest that as a rule-of-thumb, drugs with Mw greater than 300 g/mol are expected to be transformed to its amorphous state using standard process technology. In addition, Mw together with T_g predicted the dry stability of 78% of the amorphous drugs correctly.

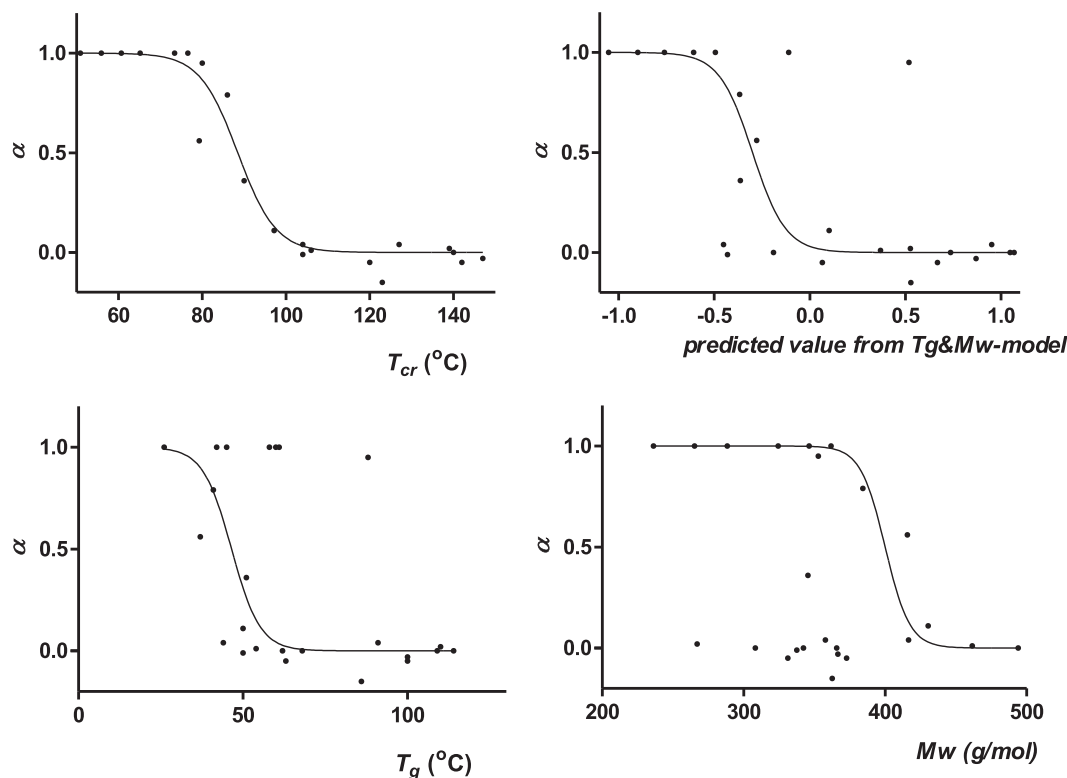


Fig. 4. Storage stability as a function of single parameters and the predictive model. The calculated fraction of the amorphous compound that had transformed after 1 month of storage at 22 °C and at dry conditions (α) plotted against T_{cr} (upper left), model predicted values based on T_g and Mw (upper right), T_g (lower left) and Mw (lower right). A strong sigmoidal relationship (R^2 of 0.96 upon fitting of Eq. (6)) to glass stability was obtained for T_{cr} showing that all compounds having $T_{cr} > 100$ °C being stable upon 1 month of storage. The other plots are shown for comparison, with line representing sigmoidal relationship inserted as a guide to the eye.

In this study we also identified a strong relationship between T_{cr} and the dry stability of the amorphous drugs. In addition to inherent compound properties, T_{cr} is sensitive to structural changes in an amorphous phase of importance for its stability, thereby being more accurate for produced amorphous materials. Taken together the findings in this study show that early stage evaluations of the inherent glass-forming ability of a compound can be made from Mw. For glass-formers, Mw together with calculated or simulated T_g can be used to predict the storage stability of the amorphous form of a drug. When an amorphous material has been produced we suggest that the T_{cr} can be used to evaluate and rationalize the selection of production technology and optimal production settings. These properties, e.g. Mw, T_g and T_{cr} , have the potential to rationalize decision-making in drug development as they help judging the potential of a compound to be formulated amorphous.

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