Applying Thermodynamic and Kinetic Parameters to Predict the Physical Stability of Two Differently Prepared Amorphous Forms of Simvastatin

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Abstract: Converting drugs from the crystalline to the amorphous state has gained increasing interest in the past decades as a potential method to overcome solubility issues of poorly water soluble drugs. A variety of techniques exist to convert the crystalline state of a drug to its amorphous form, including solution based, heat based and solid - solid conversion based methods. Inherent to the amorphous state, regardless of its preparation technique, is its physical instability and tendency to recrystallize. In this study, quench–cooled and cryo–milled simvastatin were compared with regards to their configurational thermodynamic parameters (entropy, enthalpy and Gibbs free energy) and mobility (relaxation times calculated using the Adam-Gibbs and Kohlrausch-Williams-Watts method). Stability studies showed quench–cooled simvastatin to be more stable than cryo–milled simvastatin. This was reflected in the calculated parameters although their absolute values did not agree with the stability behaviour. Relaxation time parameters of $\tau = 6.9 \cdot 10^4$ s for quench-cooled and $\tau = 1.7 \cdot 10^4$ s for cryo-milled simvastatin were calculated. The results from this study suggested that differences in the physical stability of amorphous forms prepared by different techniques are reflected in their mobility and thermodynamic parameters. Even though the predictive capabilities of these parameters for a set of different drugs may be limited, they can serve as a predictive tool for physical stability assessment if differently prepared amorphous forms of the same drug are investigated.

Keywords: Simvastatin, amorphous, physical stability, Kohlrausch-Williams-Watts, Adam-Gibbs, DSC.

1. INTRODUCTION

With the advent of combinatorial chemistry and high throughput screening, a large proportion of new compounds are highly crystalline, and their aqueous solubility frequently can be very low, jeopardizing their further development. Crystalline solids are characterized by their long range translational and orientational order. Their molecular arrangement is in defined lattices (unit cells) and highly symmetrical [1]. Amorphous solids, however, lack this long-range order although short range order over several molecular dimensions may exist [2]. The molecular arrangement of amorphous solids is thought to represent that of a frozen liquid, however with the rheological properties of a solid [3]. The amorphous state shows excess free energy, enthalpy, entropy and mobility compared to the crystalline state and therefore its solubility may be higher resulting in an increased bioavailability of the compound. Therefore, the conversion of a crystalline solid into the amorphous state has been recognized as a suitable method to overcome solubility issues of poorly water soluble drugs [1]. Dissolution rate and solubility advantages have been reported for a number of drugs including indomethacin [4], carbamazepin [5], cefalexin [6] and simvastatin [7].

Although the amorphous state has been shown to be advantageous over the crystalline state with respect to solubility, only few amorphous drugs have been marketed such as Kaletra® (ritonavir and lopinavir), Sporanox® (itraconazole) and Prograf® (tacrolimus). This is frequently due to the inherent physical instability of the amorphous state which may lead to recrystallization over time. Within a formulation, even partial recrystallization is unacceptable.

Currently, the physical stability of amorphous drugs has to be individually assessed through time consuming storage experiments. Accelerated studies are limited as it has been shown that the behaviour of amorphous drugs at higher temperatures (e.g. above their glass transition temperature) is not necessarily related to the actual stability behaviour at the intended storage temperature [8-10].

The recrystallization of the amorphous state is a complex process which is influenced by a large number of factors, among them hydrogen bonding [11], moisture uptake [12], fragility [13] and thermal history of the sample [14, 15]. The most influential factors affecting the physical stability however, have been considered to be the configurational thermodynamic parameters [16-18] and the molecular mobility [19-21].

As early as 1936, correlations between recrystallization and molecular mobility have been drawn [22]. The routine approach for quantifying molecular mobility, measured as its reciprocal, the relaxation time τ, has been to age a sample for various lengths of time at a given temperature below the T_g and to then calculate the relaxation time constants τ and β [20, 23-25]. The enthalpy that has been lost during ageing at temperatures below the T_g is estimated by measuring the enthalpy overshoot at T_g during heating of the sample. Using DSC, this enthalpy overshoot can be quantified and used to calculate the relaxation time τ. The data is fitted to the empirical Kohlrausch-Williams-Watts (KWW) equation (eq. **1**)

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and the average τ and the stretch parameter β can be obtained.

$$\varphi_{(T,t)} = \exp\left[-\left(\frac{t}{\tau}\right)^{\beta}\right] = 1 - \frac{\Delta H_{relax}}{\Delta H_{\infty}} \qquad \text{eq. (1)}$$

In this equation, ΔH_{relax} is the measured enthalpy and ΔH_{∞} represents the greatest theoretically possible enthalpy that can be recovered and is calculated as

$$\Delta H_{\infty} = \Delta Cp\left(T_g - T\right) \qquad \text{eq. (2)}$$

Though the KWW equation is commonly used, it has theoretical limitations. The most important being that the relaxation time τ is considered to be constant during the experiment. This assumption has been shown to not necessarily hold true [24, 26]. Furthermore, as the relaxation is accompanied by some degree of non-exponentiality, comparison between two τ-values may only be valid if the β-values are close [25, 27]. Kawakami *et al*. have suggested using the stretched time constant τ^{β} as an indicator of relaxation time. During the annealing experiment, τ increases with time and β decreases with time. However, τ^{β} remains relatively invariant and can therefore serve as a stability indicator [24].

The limitations of the widely used KWW equation have been discussed in detail in the literature [28] and it has been suggested to address these by employing the Adam-Gibbs (AG) equation in order to calculate the relaxation time more precisely. A detailed explanation of the theory and derivation of the equations used in this study can be found elsewhere [29-32].

In one of its forms the Adam-Gibbs equation can be written as:

$$\tau = \tau_0 \exp\left(\frac{DT_0}{T\left(1 - T_0/T_f\right)}\right) \qquad \text{eq. (3)}$$

Where τ_0 denotes a constant, taken as the lifetime of atomic vibrations, 10^{-14} s, D is Angell's strength parameter (and a measure of fragility of a glass forming liquid), T_0 is the temperature where no structural mobility occurs and T_f is the fictive temperature. The fictive temperature is the temperature at which the observed properties of a glass correspond to that of the equilibrium state. It can be calculated from the heat capacities and the T_g of the glass using equations (**4**) and (**5**).

$$\frac{1}{T_f} = \frac{\gamma_{Cp}}{T_g} + \frac{1 - \gamma_{Cp}}{T} \qquad \text{eq. (4)}$$

$$\gamma_{Cp} = \frac{Cp^{l} - Cp^{g}}{Cp^{l} - Cp^{x}} \qquad \text{eq. (5)}$$

The scanning rate dependence of the T_g has been shown to be a suitable method for the calculation of the activation enthalpy at the T_g [13] and by employing the activation enthalpy, the strength parameter D and T_0 can be calculated using the following equations:

$$\frac{\Delta H(T_g)^{*}}{R} = \frac{d\ln q}{d(1/T_g)} \qquad \text{eq. (6)}$$

$$m = \left.\frac{d\log\tau}{d(T_g/T)}\right|_{T=Tg} = \frac{\Delta H(T_g)^{*}}{(\ln 10)RT_g} \qquad \text{eq. (7)}$$

$$D = \frac{\ln 10 * m_{\min}^{2}}{m - m_{\min}} \qquad \text{eq. (8)}$$

$$T_0 = T_g\left(1 - \frac{m_{\min}}{m}\right) \qquad \text{eq. (9)}$$

Here, $\Delta H(T_g)^*$ is the activation enthalpy at the T_g, R is the gas constant, q is the heating rate and m is the fragility index. The minimum possible fragility value, $m_{min,}$ corresponds to the relaxation of the unrestricted material at T_g, and has been calculated to be 16 [26]. The strong/fragile classification system is used in amorphous sciences to characterize the temperature dependence of viscosity and mobility. The strength parameter D and the fragility index m are used in order to classify compounds according to their fragility. Fragile liquids show a strong temperature dependence of mobility, whereas strong liquids show Arrhenius-like behaviour [13].

Configurational thermodynamic properties such as the entropy and Gibbs free energy have been shown to be correlated to some extent with the physical stability above T_g [16, 33]. Zhou *et al*. proposed a method for calculating the thermodynamic properties of amorphous forms using the configurational heat capacity Cp_{conf}, obtained from DSC measurements [16].

$$H_{conf} = \Delta H_m + \int_{T_m}^{T} Cp_{conf}\,dT \qquad \text{eq. (10)}$$

$$S_{conf} = \Delta S_m + \int_{T_m}^{T} \frac{Cp_{conf}}{T}\,dT \qquad \text{eq. (11)}$$

$$\Delta S_m = \frac{\Delta H_m}{T_m} \qquad \text{eq. (12)}$$

In these equations ΔH_m and ΔS_m are the melting enthalpy and entropy respectively.

The temperature dependence of the Cp_{conf} has been shown to follow the relationship described in eq. (**13**) [16]:

$$Cp_{conf} = \frac{K}{T} \qquad \text{eq. (13)}$$

with K being a constant.

Determination of K allows the calculation of the Kauzmann temperature, T_K, which is the lowest theoretically possible glass transition temperature at which molecular mobility of an amorphous material should become negligible.

$$\frac{1}{T_K} = \frac{1}{T_m}\left(1 + \frac{\Delta H_m}{K}\right) \qquad \text{eq. (14)}$$

It is often assumed that the T_K lies 50 ºC below the T_g, however, calculation provides a more precise value as it has been shown that the 'T_g – 50 ºC rule' does not necessarily apply.

It is known that amorphous states prepared using different techniques may show differences in their physical stability [34-36]. These differences should be reflected in differing

values for the relaxation time and configurational thermodynamic parameters of differently prepared amorphous samples. In this study, simvastatin was used as a model compound. The properties of the amorphous form have been shown to be preparative technique dependent [37]. The preparative techniques used were quench-cooling of the melt and cryo-milling. These methods will result in amorphous states that have different thermal histories. For this study it was also of interest, to investigate how the common procedure of erasing the thermal history of a sample by heating and holding it above its T_g may affect the amorphous state that had not been prepared by a heat based method.

Comparison of predictive parameters for different amorphous preparative approaches on the same drug may provide a deeper insight into their stability behaviour and enable an early decision on the usefulness of possible methods to formulate amorphous forms of a given drug.

It has been shown that the calculated relaxation time τ is not necessarily directly correlated to physical stability below T_g [33], as the aforementioned factors will affect stability to differing extents. However, even though general conclusions for different compounds are difficult to draw, we hypothesize that these parameters will correlate with the stability behaviour of the same compound prepared in different ways.

2. MATERIAL AND METHODS

Materials

Simvastatin Fig. (**1**) was used as received from Biocon Laboratories, Bangalore, India.

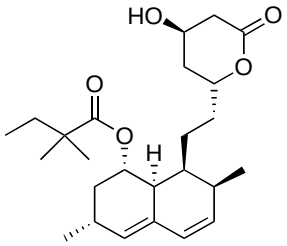

Fig. (1). Chemical structure of simvastatin.

Preparative Techniques

Cryo-Milling

Simvastatin was ball milled in a Retsch Mixer Mill MM300/301 (Retsch, Haan, Germany) using a 25 ml stainless steel chamber for 90 min with 2 x 12 mm diameter and 6 x 7 mm diameter stainless steel balls. The samples were milled at 30 Hz. The milling process was carried out under cryogenic conditions (the chambers were covered with liquid nitrogen for 2 minutes at 15 minute intervals to ensure a sufficiently low milling temperature).

Melting and Quench-Cooling

Quench-cooled simvastatin was prepared by melting and cooling in the DSC instrument (Q100, TA-Instruments-Waters LLC, New Castle, USA). For each sample, approximately 5 mg of simvastatin was heated to 150 ºC at a heating rate of 10 K/min, held at that temperature for 4 minutes and subsequently cooled to -20 ºC at a cooling rate of 20 K/min.

Analytical Techniques

(i) XRPD

Crystallinity was determined using a PANalytical X'Pert Pro MPD diffractometer (Eindhoven, Holland). CuKα (wavelength 1.5406 Å) radiation was used at 40 kV and 30 mA. Samples were scanned from 5 – 40º 2θ. The diffractometer was calibrated using powdered α-aluminium oxide. Results were analyzed using the X'Pert Data Viewer.

(ii) HPLC

Analysis was carried out on an Agilent 1100 series HPLC (Agilent Technologies, Waldbronn, Germany) using a Phenomenex Luna 3 µm C18, 150 x 4.6 mm column and a UV detector. An isocratic solvent system consisting of 20 % (v/v) water and 80 % (v/v) acetonitrile was used at a flow rate of 1.0 ml/min. Both mobile phases contained 0.05 % (v/v) TFA. The column temperature was 40 ºC and the chromatograms were analyzed at uv_{max} = 238 nm. Samples were dissolved in acetonitrile. All analyses were carried out in triplicate.

(iii) DSC

A TA Instruments Q100 (TA-Instruments-Waters LLC, New Castle, USA), equipped with an RCS cooling system was used. Nitrogen was used as purge gas at 50 ml/min. The instrument was calibrated in standard mode for melting temperature using indium and in the modulated mode for heat capacity using sapphire. A modified procedure of calibration was used according to Hill *et al.* [38]. The suitability of the calibration and the experimental conditions were checked by comparison to the literature values of dry crystalline sucrose [39].

(a) Configurational Thermodynamic Properties

The quench-cooled samples were prepared *in situ* in the DSC instrument. In order to minimize heat transfer effects due to weight differences, geometry of the sample or packing in the DSC pan, the same sample was used for determining the heat capacity in the crystalline and the quench-cooled amorphous state. After measurement of the crystalline heat capacity, the same sample was melted, quench cooled in the DSC and another run was carried out to determine the amorphous properties. For the cryo-milled samples, a fresh sample was used for each heat capacity measurement. Configurational heat capacity measurements were carried out in temperature modulated mode (MTDSC) at a heating rate of 1 K/min using an amplitude of ± 0.5 K and a modulation period of 100 s. The reversing heat capacity was obtained by deconvoluting the total heat capacity using TA Universal Analysis 2000 Software.

Calculations of the configurational free energy, enthalpy and entropy were carried out using a MatLab software programme (MatLab R2008a, The MathWorks Inc., Natick, MA, USA).

(b) Kinetic Properties

Kohlrausch-Williams-Watts

Amorphous samples were obtained by heating the drugs 5 ºC above their melting temperature and holding them for 5

min at that temperature to ensure complete melting. The drugs were then cooled at a cooling rate of 20 K/min to the ageing temperature at approximately 20 ºC below their respective T_g and held at that temperature for 0, 2, 4, 8, 12 and 16 h. The relaxation enthalpy was determined by re-heating the samples through their T_g in conventional DSC mode and measuring the relaxation endotherm at T_g.

Adam Gibbs

The scanning rate dependence of the glass transition was measured at heating rates of 2, 5, 10, 15 and 20 K/min. The glass transition temperature was taken as the inflection point of the step change. The quench-cooled samples were prepared in the DSC instrument. Samples were melted, subsequently cooled to – 20 °C, and equilibrated at that temperature for 4 min. The amorphous drug was then heated through its T_g at a certain heating rate (2, 5, 10, 15 or 20 K/min) to 60 °C. The sample was then cooled back to the starting temperature at the same rate and then heated once more. Measurements were taken from the second heating run. All measurements were performed in triplicate.

The cryo-milled samples were not pretreated. The sample was placed in the DSC instrument and heated at a certain heating rate (2, 5, 10, 15 or 20 K/min). The glass transition temperature was taken from that heating run.

Stability Below T_g

Approximately 3 – 5 mg of cryo-milled simvastatin was placed in DSC pans prior to storage. The quench-cooled samples were prepared in the DSC and then transferred to their storage conditions. Samples were stored at 5 ºC and 0 % RH for the quench-cooled samples and 0 ºC and 0 % RH for cryo-milled simvastatin, which related to storage approximately 20 ºC below their respective T_gs. Measurements were carried out over a period of 30 days at regular intervals and the amorphous content of the samples was assessed by relating the measured recrystallization enthalpy of the samples to the recrystallization enthalpy of the fresh sample (all measurements were carried out in triplicate).

3. RESULTS AND DISCUSSION

Investigation into the Thermal Behaviour of Differently Treated Amorphous Samples of Simvastatin

Analysis of the quench-cooled (QC) and cryo-milled (CM) simvastatin confirmed previous results regarding differences between the two amorphous materials in the T_g and the recrystallization temperature, T_c (Fig. **2**) [37].

The cryo-milled simvastatin showed a significantly lower T_g and recrystallized upon heating in the DSC, whereas the quench-cooled simvastatin remained amorphous (Table **1**). The cryo-milled simvastatin showed no sign of a relaxation endotherm directly after preparation, whereas the quench-cooled samples showed a small endotherm of 0.53 J/g.

Comparison of the cryo-milled (CM) with the cryo-milled history erased (CM-hist) samples showed significant differences in all properties apart from the recrystallization enthalpy and subsequent melting of the crystal form (H_c, T_m and H_m) as shown in Table **1**.

The most pronounced differences observed were the increase in glass transition temperature by 8.3 ºC from 20.6 to 28.9 ºC. Due to the kinetic nature of the T_g, different preparation techniques may lead to different values for the glass transition temperature. In this study the original T_g of 20.6 ºC for the CM simvastatin was replaced by a T_g of 28.9 ºC when the sample was heated and cooled, indicating that the heating and cooling procedure had changed the CM properties.

Erasing the milling history of the CM sample by holding it above its T_g followed by cooling and re-heating at a defined rate imposes a thermal treatment on the sample which is reflected by a change in T_g. The resultant T_g appears similar to the quench-cooled T_g (Fig. **2**). A slightly lower heat capacity of the history erased sample is observed, however comparison of the recrystallization enthalpies and PLM could not detect any crystallization. The standard deviation of the recrystallization enthalpy was larger for the history erased sample than for the untreated sample which could also be attributed to some recrystallization. However, the history erased form showed a higher recrystallization temperature which indicated a slightly more stable amorphous form compared to the untreated cryo-milled simvastatin. This could be treated as an indication that the CM sample had taken on properties of the QC sample after thermal treatment. This had already been observed by Luthra *et al*. where lyophilized aspartame:trehalose mixture showed different DSC thermograms after the thermal treatment of formulation [40].The QC sample did not recrystallize under these conditions.

After erasing the history of the cryo-milled simvastatin, a small relaxation endotherm was measured which had not been observed in the untreated sample. A relaxation endotherm was observed for the QC simvastatin but not for the CM simvastatin. It may be hypothesized that amorphous regions already started to relax during the milling process and after 90 min a completely relaxed amorphous sample had been created. The following thermal treatment on the

Table 1. Thermal Properties of QC, CM and CM-hist Simvastatin

Method	T_g [ºC]	±s.d.	Cp [J/gºC]	±s.d.	H_{relax} [J/g]	±s.d.	T_c [ºC]	±s.d.	H_c [J/g]	±s.d.	T_m [ºC]	±s.d.	H_m [J/g]	±s.d.
CM	20.6*	1.0	0.393*	0.014	/		45.6*	3.6	34.4	4.5	135.2	0.3	61.81	2.1
CM hist	28.9*	1.8	0.334*	0.027	0.36*	0.17	57.8*	1.2	38.3	5.09	135.4	0.3	66.9	9.8
QC	31.9	0.1	0.375	0.02	0.53	0.07	n/a		n/a		n/a		n/a	

*=significantly different between CM and CM-hist, $p<0.05$; n = 3. Cp represents the heat capacity, H_{relax}, H_c and H_m are the relaxation, crystallization and melting enthalpy respectively and T_c and T_m the crystallization and melting temperatures. n/a refers to no recrystallization of the amorphous sample.

CM sample resulted in the sample residing in the super–cooled liquid state and attaining sufficient mobility for molecular rearrangement. Subsequent cooling led to the creation of a glassy state that had not been able to relax during its preparation and therefore showed a relaxation endotherm at the T_g. Its value of 0.36 J/g was not significantly different from that of the QC simvastatin (0.53 J/g) and suggested that applying thermal energy may induce mobility.

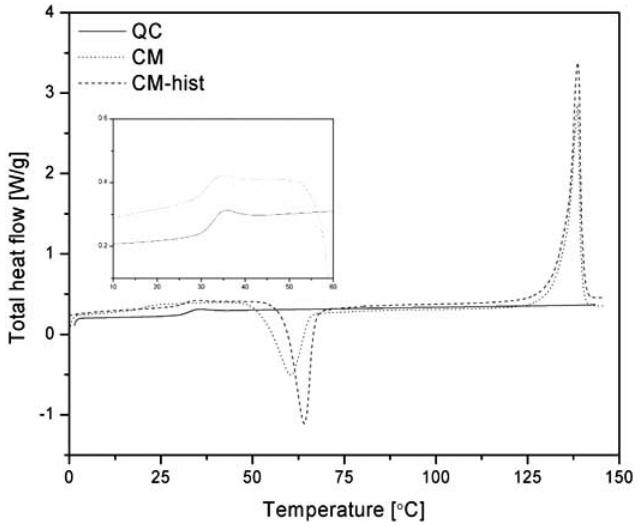

Fig. (2). Glass transition temperatures and recrystallization behaviour of CM, CM-hist and QC simvastatin. The inset shows an enlargement of the glass transition region.

From these results it can be concluded, that thermally erasing the history of an amorphous sample, which was not prepared by heating and cooling, will result in an amorphous sample with properties closer to a quench-cooled sample.

Physical Stability of QC and CM Simvastatin During Storage

The two differently prepared amorphous samples exhibited different stability behaviour upon storage at approximately 20 ºC below their respective T_gs. The amorphous content of both amorphous forms during storage is presented in Fig. (**3**) and it is evident that CM simvastatin exhibited a faster onset of recrystallization. QC simvastatin showed detectable recrystallisation after 21 days of storage whereas CM simvastatin recrystallized after 1 day under similar storage conditions.

The differently prepared amorphous forms showed different physical stability behaviour. It may be hypothesized that these different properties are reflected in different values for the mobility and thermodynamic parameters.

Comparison of Stability Indicators for Quench-Cooled And Cryo-Milled Simvastatin

Thermodynamic Parameters

The configurational thermodynamic parameters were calculated for both amorphous forms of simvastatin. The results showed that there were marked differences in the thermodynamic properties of QC and CM simvastatin. An overview over the parameters is given in Table **2**.

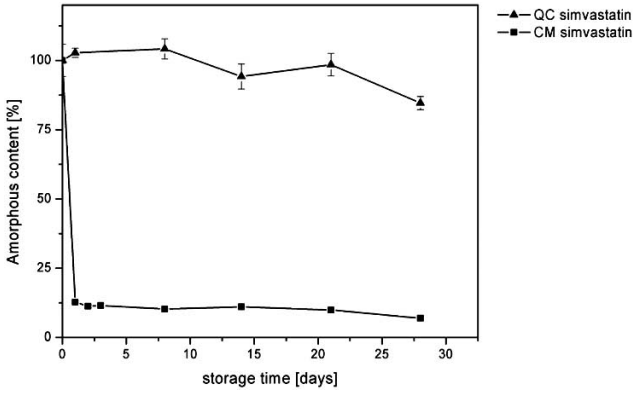

Fig. (3). Amorphous content of QC and CM simvastatin after storage for 30 days.

The Kauzmann temperature (T_K) was lower for CM than for QC simvastatin and the difference between the T_g and the

Table 2. Thermal and Thermodynamic Parameters for Two Differently Prepared Amorphous Forms of Simvastatin

	Preparative Technique	
	Quench-cooled	**Cryo-milled**
T_g [K]	305	294
Cp [J/gK]	0.375	0.393
T_K [K]	244	214
T_g-T_k	61	80
S_{conf} [J/molK]	56.2	59.1
H_{conf} [kJ/mol]	24.5	25.4
G_{conf} [kJ/mol]	4.5	6.7

T_K was larger. A temperature difference of approximately 50 ºC between the glass transition temperature and T_K is commonly assumed [41] and the larger difference found for CM simvastatin indicated that it may show excess mobility and may be less stable then the QC simvastatin. The CM simvastatin would have to be cooled to a lower temperature before mobility becomes negligible.

The configurational parameters S_{conf} and H_{conf} were found to be slightly higher for CM than for QC simvastatin. The overall driving force for recrystallization (represented by G_{conf}) was higher for the CM simvastatin, suggesting that CM simvastatin should recrystallize more readily than QC simvastatin.

Kinetic Parameters

Relaxation Time (Kohlrausch – Williams –Watts Equation)

Molecular mobility was assessed through application of the KWW equation. Analysis of the relaxation endotherms revealed different behaviour for the two differently prepared amorphous forms (Fig. **4**).

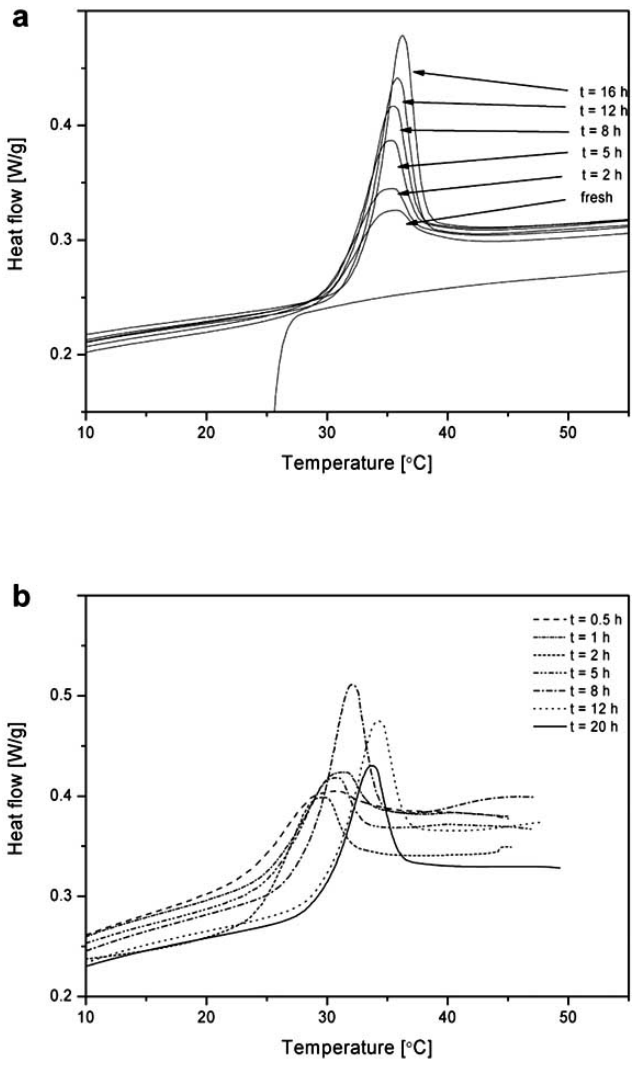

Fig. (4). Relaxation endotherms and temperature evolution of the glass transition temperature for a) QC and b) CM simvastatin.

The QC simvastatin showed a small increase of the glass transition temperature during annealing (ΔT_g = 0.9 ºC). This value is in accordance with values for other glasses [24]. For the CM simvastatin, however, an increase of the temperature of the glass transition of ΔT_g = 4.7 ºC was detected. An effect of ageing time on the T_g has been reported [42] [43] and has to be taken into account when estimating the relaxation time. The initial excess enthalpy H_∞ was calculated using the T_g (eq. **2**), hence if the glass transition temperature increased during annealing, the calculated initial excess enthalpy will be determined incorrectly.

It has been suggested to account for the overestimation of H_∞ by applying a correction term [24] (eq. **15**):

$$H_\infty(t,0) - H_\infty(t,T) = \Delta H_{relax} - \Delta Cp\Delta T_g(t,T) \qquad \text{eq. (15)}$$

Here, $\Delta T_g(t,T)$ represents the change in T_g during annealing. Eq. (**15**) can be used to calculate the relaxation function φ taking the increase of T_g into account (eq. **16**):

$$\varphi(t,T) = 1 - \frac{H_{relax}(t,T) - \Delta Cp\Delta T_g(t,T)}{\Delta Cp(T_g - T)} \qquad \text{eq. (16)}$$

The effect of the increase of T_g during annealing on the relaxation function φ can be seen in Fig. (**5**), where the relaxation function φ was fitted to the KWW equation for CM simvastatin, without and with correction for the T_g.

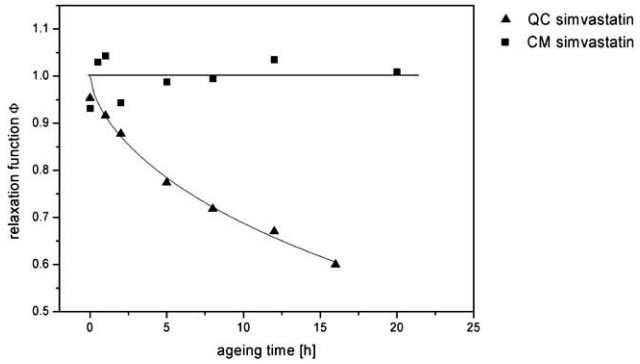

Fig. (5). Effect of temperature correction for T_g on the relaxation function φ of CM simvastatin. The line at φ = 1 does not represent a fit to the KWW equation but aids to visualize the distribution of values around 1.

It was evident that the T_g correction significantly impacted on the results of the annealing experiment for CM simvastatin. The uncorrected relaxation function led to erroneous results for τ ($3.1 \cdot 10^7$ s) and β (0.3). In contrast, the corrected values could not be fitted to the KWW equation (details see below). Comparison of the relaxation functions of CM and QC simvastatin and their fit to the KWW equation is presented in Fig. (**6**).

The QC simvastatin showed a good fit of the relaxation function φ to the KWW equation, resulting in values for τ of $2 \cdot 10^5$ s and β of 0.5. As stated above, the relaxation function values for CM simvastatin showed no time dependence and were close to 1. A relaxation function value of 1 is obtained if the sample has relaxed completely and the value for ΔH_{relax} is 0. This resulted in the KWW not being able to attribute a relaxation time constant value for the CM simvastatin. Al-

though there was an apparent increase in the relaxation endotherm upon storage of CM simvastatin, the temperature dependence of the T_g has to be taken into consideration. Correction of the values led to the conclusion that CM simvastatin had already relaxed almost completely over the duration of the experiment. Although the CM simvastatin had apparently reached a maximum degree of relaxation, no indication of recrystallization could be detected over the period of annealing.

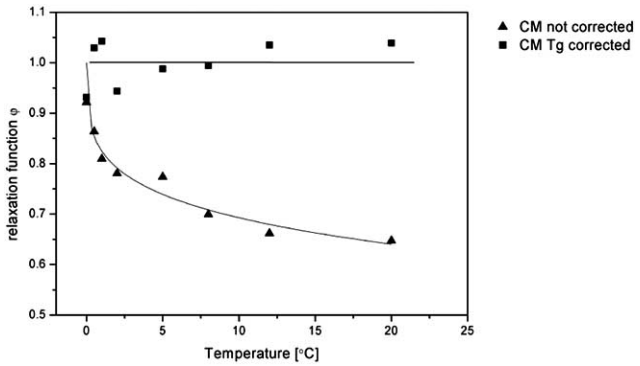

Fig. (6). The relaxation function φ as a function of annealing time and fit to the KWW equation for QC and CM simvastatin. The line at φ = 1 does not represent a fit to the KWW equation but aids to visualize the distribution of values around 1.

Relaxation Time (Adam-Gibbs Equation)

The glass transition temperature values changed differently as a function of heating rate for the differently prepared amorphous forms (Fig. **7**). The QC simvastatin showed a stronger temperature dependence than the CM simvastatin which resulted in the QC simvastatin exhibiting a larger activation enthalpy at T_g [$\Delta H^*(T_g)$].

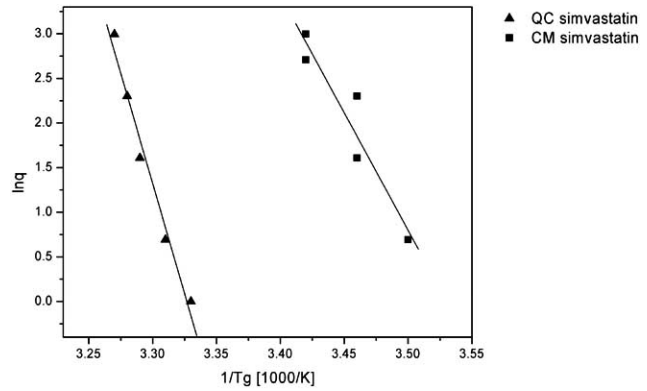

Fig. (7). Arrhenius plot of the heating rate dependence of QC and CM simvastatin. The r^2 values were 0.963 and 0.940 respectively.

It has been proposed that a larger value of $\Delta H^*(T_g)$ is typical of fragile glass forming liquids due their non-Arrhenius behaviour close to T_g [13, 44], which indicated that the CM simvastatin may be a stronger glass former than the QC simvastatin.

Calculation of the kinetic parameters was carried out based on the activation enthalpy and the values are presented in (Table **3**). The fragility parameters D and m clearly suggested that there was a large difference in fragility between the two differently prepared amorphous forms: CM simvastatin appeared to be much less 'fragile' than the QC simvastatin and showed values that were close those of 'strong' liquids (fragile liquids: $D < 10$, $m < 200$; strong liquids: $D > 30$, $m \approx 16$).

Increased mobility of the CM simvastatin could be detected which was reflected in the lower values for T_0. The temperature at which the structural mobility can be considered negligible was lower, indicating that CM samples would need to be stored at lower temperatures than QC simvastatin.

Table 3. Kinetic Properties of QC and CM Simvastatin

	Preparative technique	
	Quench-cooled	**Cryo-milled**
$\Delta H^*(T_g)$ [kJ/mol]	412	215
D	9.5	26.5
m	78.2	28.3
T_0 [K]	243	171
γCp	0.81	0.40
τ (KWW) [s]	$1.7 \cdot 10^5$	n.a.
τ (AG) [s]	$6.9 \cdot 10^4$	$1.7 \cdot 10^4$

The relaxation time values at a temperature of 20 ºC below the respective T_gs supported this. QC simvastatin exhibited a relaxation time constant of $6.9 \cdot 10^4$ s (19 h) and CM simvastatin showed a value of $1.7\ 10^4$ s (5 h) calculated using the AG equation. The higher mobility of CM simvastatin at T_g – 20 ºC is reflected in the lower stability and faster onset of recrystallization upon storage.

The relaxation time constant for the QC simvastatin calculated using the AG equation was lower than the value calculated using the KWW approach which gave a value of $\tau = 1.7 \cdot 10^5$ s which equals to 47 h. This was not unexpected, as both equations are based on different underlying considerations. The KWW approach led to an average relaxation time value, based on enthalpic relaxation of the glassy state. The AG concept comprises the excess entropy that is present in a glass and influences mobility. The absolute values for the relaxation times did not agree with the measured physical stability of the QC amorphous form, and the calculated values underestimated the stability. The CM simvastatin showed a relaxation time value of 5 h, and after the first day of storage the sample had started to recrystallize, indicating the reduced stability of CM simvastatin. However, the values for the relaxation times reflected the tendencies of stability for the differently prepared amorphous forms. The CM simvastatin showed the lower relaxation time compared to the QC simvastatin and was the less stable of the two amorphous forms.

CONCLUSION

This study was intended to provide further insight into the different behaviour of differently prepared amorphous

forms. It could be shown that the properties of an amorphous state depended on its preparation technique and the amorphous state was susceptible to changes post preparation. Thermal treatment of a non-thermally prepared amorphous form (cryo-milling) resulted in the loss of the amorphous characteristics of the milled form**.** Therefore, when dealing with differently prepared amorphous forms care has to be taken if their further analysis or further processing steps involve thermal treatment.

Furthermore, comparison of two differently prepared amorphous forms of simvastatin showed differences in their physical stability and this was reflected in their thermodynamic and kinetic properties. CM simvastatin was found to have 'strong' glass forming properties, whereas QC simvastatin was shown to be a fragile glass former. The relaxation time and thermodynamic parameters differed for the amorphous states and showed CM simvastatin to have a larger mobility and thermodynamic driving force for recrystallization. The differences in the thermodynamic parameters between the two differently prepared amorphous forms were not pronounced, indicating that for the same compound thermodynamic characteristics may only vary slightly. The absolute values for the relaxation time did not agree with the observed stability, however, the relative differences served as a tool to determine the enhanced physical stability of the QC simvastatin over the CM simvastatin. Additional studies are needed to confirm the capability of the relaxation time in predicting the stability of differently prepared amorphous forms of the same compound, however, the results from this study appear promising.

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