*Journal of Thermal Analysis and Calorimetry, Vol. 77 (2004) 523–530*

# DSC ANALYSIS OF THE ANTI-HIV AGENT LOVIRIDE AS A PREFORMULATION TOOL IN THE DEVELOPMENT OF HOT-MELT EXTRUDATES

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## Abstract

Thermal analysis was performed on the anti-HIV agent loviride in order to test its suitability to be processed using hot-melt extrusion. Temperature characteristic parameters of crystallization were determined to quantify the stability of amorphous loviride. The present study has shown that cooling and heating loviride at different rates influenced its thermal stability. At high cooling rates melted loviride did not crystallize during cooling, and formed a glass that recrystallized during reheating. Very low cooling rates resulted in significant decomposition of the drug. The glass transition temperature was found to increase as a function of increasing heating rates and the activation energy for the transition from the glassy to the super-cooled liquid state was relatively high, indicating good stability of the glass.

**Keywords**: crystallization, differential scanning calorimetry, glass transition temperature, loviride, thermal stability

## Introduction

Loviride (Fig. 1) is a potent, non-nucleoside reverse transcriptase inhibitor (NNRTI) with an $IC_{50}$ value of 9 nM against HIV-1 and a cytotoxic potential ($CC_{50}$) of >350 μM (MT-4 cells) giving an in-vitro therapeutic index of >39.000 [1]. Despite the potential of this compound, its poor aqueous solubility and low dissolution rate limit its oral absorption. A strategy that is currently used to improve the biopharmaceutical performance of poorly soluble drugs is the formulation of solid/molecular dispersions. The term refers to a dispersion of one or more active ingredients in an inert and hydrophilic carrier or matrix in the solid-state [2, 3]. The distribution of the drug in the carrier, in some cases at molecular level, together with the enhanced wettability and the micro-environment created by the carrier may increase the dissolution rate and solubility. The use of hot melt

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*1388–6150/2004/ $ 20.00*

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extrusion was recently introduced in the pharmaceutical industry but it can already be considered as one of the most promising technologies in the manufacturing of solid dispersions [4, 5]. However, one of the requirements to use hot melt extrusion is the thermal stability of the drug. Moreover, one should always be aware of the inherent metastability of the amorphous state created during hot stage processing. It is therefore necessary to have a preformulation tool available which enables to quantify both the chemical stability and the liability of the glassy state of the drug substance to recrystallization.

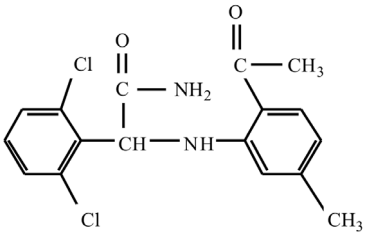


**Fig. 1** Structure of loviride

Intrinsic stability of the glassy state can be studied using the mean relaxation time constant and distribution parameter based on enthalpy recovery measurements [6–9]. Despite the merit of this procedure, we recently introduced an alternative for this time consuming method [10], based on the activation energy associated with the transition from the glassy to the super-cooled liquid-state. Other parameters to predict the stability of glasses or amorphous compounds that have been described rely on the temperature difference between the glass transition and the crystallization transition or are based on the crystallization activation energy and rate constant [11–13].

The aim of the present paper is to report on the assessment of the thermal stability of loviride by using a set of conventional DSC measurements on the crystalline and amorphous form of the drug. The procedure presented in this paper may serve as a rapid tool to assess a drug substance for its suitability in a hot melt extrusion process.

## Materials and methods

### *Materials*

Loviride was provided by Janssen Pharmaceutica (Beerse, Belgium).

### *Thermal analysis*

Influence of cooling rate

In order to investigate the influence of the cooling rate on the recrystallization behavior of liquid (melted) loviride, differential scanning calorimetry (DSC) measurements were carried out using a PerkinElmer DSC-7 differential scanning calorimeter (PerkinElmer, Norwalk, CT, USA) equipped with a liquid nitrogen subambient ac-

cessory (PerkinElmer, Norwalk, CT, USA). The instrument operated under nitrogen purge gas at a rate of 20 mL $min^{-1}$. Octadecane and indium were used to calibrate the DSC temperature scale while enthalpic response was calibrated with indium. Daily validation using the same standard materials showed that deviation of the experimental value from the theoretical one was less than 0.5 K for the temperature measurement and less than 1.0% for the enthalpy measurement. Samples (mass range 2.50–5.00 mg) were analysed in hermetically sealed aluminium pans (TA Instruments, Leatherhead, UK). Data were treated mathematically using the Pyris software version 3.6 (PerkinElmer, Norwalk, CT, USA).

The influence of the cooling rate on the crystallization behaviour of liquid loviride was determined by cooling the melt through its glass transition temperature at different rates ranging from 1 to 40 K $min^{-1}$. Subsequently the glassy material was reheated at 10 K $min^{-1}$ to 10 K above the melting point of the crystalline material.

### Influence of heating rate

Measurements at different heating rates, were performed on a Mettler-Toledo DSC822[e] equipped with an intercooler (Mettler-Toledo, Switzerland). The instrument operated under nitrogen purge gas at a rate of 20 mL $min^{-1}$. To calibrate the temperature scale and enthalpic response, mercury and indium were used. The calibration of temperature and enthalpy was validated daily using the same standard materials. At least one randomly chosen sample was heated at a randomly chosen heating rate. Deviation of the experimental value from the theoretical one was less than 0.3 K for the temperature measurement and less than 2.0% for the enthalpy measurement. Samples (mass range 2.50–5.00 mg) were also analysed in hermetically sealed aluminium pans (Mettler-Toledo, Switzerland). The results were analysed with the STAR[e] software version 6.0 (Mettler-Toledo, Switzerland).

In order to investigate the influence of the heating rate on the position of the $T_g$ and the cold crystallization behaviour of glassy loviride, samples of pure crystalline loviride were heated to 10 K above its melting point at 10 K $min^{-1}$ and cooled at 100 K $min^{-1}$ to 40 K below its $T_g$. Several temperature parameters, such as the glass transition temperature ($T_g$), the onset of crystallization ($T_x$), the crystallization peak temperature ($T_p$), the onset of melting ($T_m$), the peak temperature of melting and the enthalpy of fusion ($\Delta H_f$) of crystalline loviride were determined in the subsequent heating run in which the heating rate was varied from 1 to 20 K $min^{-1}$. The $T_g$ was calculated according to the Richardson method ($T_{g\,r}$) [14] as well as the half height heat flow method ($T_{g\,1/2\,cp}$ = the temperature at half the height of the shift in the heat flow signal), which is the most common method for the determination of the $T_g$ used in the pharmaceutical field. The inflection point temperature of crystallization ($T_f$) was obtained from the derivative differential thermal (DDT) curves of recrystallized loviride. DDT curves were obtained by differentiating the DSC curves and the inflection point temperature was determined as the peak temperature on the DDT curves.

## Results and discussion

*Influence of the cooling rate on the thermal stability of liquid loviride*

Cooling of liquid loviride at different rates ranging from 1 to 40 K $min^{-1}$, showed no crystallization during cooling. Typical DSC heating curves obtained after cooling at 1 and 40 K $min^{-1}$ are presented in Fig. 2. A clear $T_g$ is observed at approximately 336 K. The change in thermal behaviour observed in this subsequent reheating step, is due to the initial cooling rate, since the reheating step of loviride was made at the same rate, i.e. 10 K $min^{-1}$. The effect of the initial cooling rate on the onset of melting, the melting peak and the enthalpy of melting in the subsequent reheating step, is shown in Table 1. At a cooling rate of 40 K $min^{-1}$, loviride showed a melting temperature of 495.6 K. When using a cooling rate of 1 K $min^{-1}$, a melting temperature of only 486.8 K was found. Not only the onset temperature ($T_m$), but also the melting peak (temperature of the maximum of the remelting peak) and the enthalpy of fusion were affected in the same manner by changing the initial cooling rate. The reduction in enthalpy of fusion of loviride, the broadness of the peaks, the low peak temperatures and onset of melting during the re-

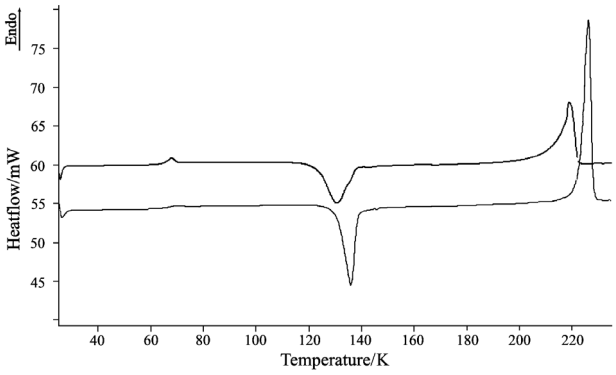


**Fig. 2** Heating curves (10 K $min^{-1}$) of glassy loviride after cooling from the melt at 1 and 40 K $min^{-1}$

**Table 1** The effect of cooling rate on the peak properties of remelted loviride

| Cooling rate/K $min^{-1}$ | Onset of remelting/K | Remelting peak*/K | Enthalpy of remelting/J $g^{-1}$ |
|---|---|---|---|
| 40 | 495.6±0.5 | 498.9±0.4 | 127.3±0.9 |
| 20 | 495.7±0.2 | 498.8±0.4 | 123.6±1.3 |
| 10 | 494.7±0.8 | 498.0±0.6 | 124.1±1.7 |
| 5 | 494.2±0.6 | 497.5±0.1 | 117.3±1.7 |
| 1 | 486.8±2.9 | 491.1±1.1 | 89.3±4.9 |

Conditions: samples of liquid loviride were cooled at different cooling rates and reheated at a rate of 10 K $min^{-1}$ to 508.15 K. For the calculation of the standard deviation at least 3 measurements were used ($n \geq 3$).

heating step after cooling at a low rate, indicate the presence of degradation products. The degradation products act as impurities and hence melting point depression results. In order to have an idea on the extent of impurity of the loviride melts, the following equation which is derived from the van't Hoff equation was used [15, 16]:

$$T_F = T_0 - \frac{RT_0^2 x}{\Delta H_0} \frac{1}{F}$$

In this equation $T_F$ represents the sample temperature at equilibrium (the temperature corresponding to the melting of a certain fraction of the total sample), $T_0$ is the melting point of the pure sample, $R$ the universal gas constant, $x$ the mole fraction of impurity, $\Delta H_0$ the enthalpy of fusion of the pure compound and $F$ the fraction of sample melted at $T_F$. The mole fraction of impurities obtained at cooling rates of 40, 20, 10, 5 and 1 K min$^{-1}$ were 0.0063, 0.0083, 0.0097, 0.0099 and 0.0216, respectively. The results show that the samples obtained at lower cooling rates have a higher concentration of impurities, which results into a melting point depression. The use of a low cooling rate leads to a prolonged residence time of the drug at high temperatures. The fact that this leads to chemical degradation of loviride indicates its relatively poor thermal stability. Hence, when processing loviride by means of hot melt extrusion, the residence time in the extruder should be kept as short as possible.

*Influence of the heating rate on the stability of loviride glass*

The relationship between the heating rate and the glass transition temperature can be represented by [17]:

$$\ln\beta = C' - \frac{E_a}{RT_g}$$

In this equation β represents the heating rate, $C'$ is a constant, $R$ the universal gas constant, $E_a$ the activation energy for the transition from the glassy to the super-cooled liquid-state, and $T_g$ the apparent glass transition temperature measured at the heating rate β. Figure 3 shows a plot of the logarithm of the heating rate *vs.* the reciprocal of the $T_g$ determined by the half height heat flow method and the reciprocal of the $T_g$ calculated according to the Richardson method. The $T_g$ increases when the heating rate increases and the linear behaviour of the plots shows that simple first order kinetics can be used to describe the transfer from the glassy to the super-cooled liquid-state [17]. In the first case, the activation energy was calculated to be 638.82 kJ mol$^{-1}$. In the second case, when the Richardson method was used, the activation energy was equal to 943.17 kJ mol$^{-1}$. It is evident that a high $E_a$ value indicates a good physical stability of the glass with respect to devitrification. When enthalpy relaxation is important, the Richardson method is more accurate from a thermodynamic point of view, since the use of the half height heat flow method leads to an overestimation of the $T_g$ [10]. Compared to other glassy pharmaceuticals that were studied, loviride ranks among those with a relatively high value of $E_a$ [10, 18].

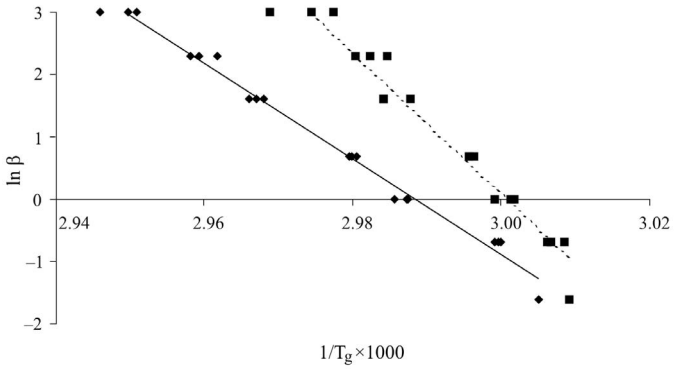


**Fig. 3** Plot of log heating rate *vs.* reciprocal of the apparent glass transition temperature of glassy loviride (◆ – $T_{g\ 1/2\ cp}$; ■ – $T_{g\ r}$; ---- linear fit through ■; —— linear fit through ◆)

The onset and peak temperature of recrystallization and the melting temperature of recrystallized loviride increased when the heating rate increased as shown in Table 2. In order to quantify this process and hence to evaluate the stability of amorphous loviride once it has passed through the $T_g$ and enters the super-cooled liquid-state, characteristic temperature criteria and crystallization activation energy were determined (Table 2). The simplest characteristic temperature criterion is:

$$\Delta T = T_x - T_g$$

where $T_x$ is the onset of crystallization and $T_g$ is the glass transition temperature. Extrapolation to zero heating rate shows that $\Delta T$ remains well above 30 K indicating a fairly high safety margin to prevent recrystallization.

Alternatively, Hruby's criterion ($H_r$), which can also be used in the case of recrystallization, is defined as

$$H_r = \frac{T_p - T_g}{T_m - T_p}$$

where $T_m$ is the melting temperature and $T_p$ is the crystallization temperature (maximum peak temperature). A modification of the $H_r$ criterion has been described, i.e. $H'$ which is defined as

$$H' = \frac{T_x - T_g}{T_g}$$

The stability criterion parameters based on the characteristic temperatures are listed in Table 2. The values of $\Delta T$, $H_r$, $H'$ indicate that the amorphous loviride crystallizes more easily at lower heating rates compared with higher heating rates.

The crystallization activation energy was calculated using the following equation [11]:

$$\ln\frac{(T_f)^2}{\beta} = \frac{E_a}{RT_f} + \ln\frac{E_a}{R} - \ln\nu$$

In this equation $T_f$ represents the inflection point temperature, $E_a$ is the crystallization activation energy, $R$ the molar gas constant, β the heating rate and the frequency factor. From a plot of $\ln(T_f)^2/\beta$ *vs.* $1/T_f$, the activation energy was calculated to be 129.1 kJ mol$^{-1}$ and the frequency factor (ν) was 3.2 ·10$^{18}$ s$^{-1}$. The crystallization activation energy can also be used to predict the stability of a glass using $E_a/RT_p$ as the determining parameter ($R$ = molar gas constant). The higher the value of $E_a/RT_p$, the higher the tendency to crystallization [19]. The results are also listed in Table 2 and are consistent with those obtained by the temperature criteria.

**Table 2** Characteristic parameters of loviride

| β/ K min$^{-1}$ | $T_{g1/2\,cp}$/ K | $T_{gr}$/ K | $T_x$/ K | $T_p$/ K | $T_m$/ K | $T_f$/ K | $\Delta T$ | $H_r$ | $H'$ | $E_a/RT_p$ |
|---|---|---|---|---|---|---|---|---|---|---|
| 20 | 339.3 | 336.3 | 405.7 | 413.9 | 496.3 | 410.4 | 66.4 | 0.91 | 0.20 | 37.5 |
| 10 | 338.4 | 335.9 | 397.6 | 404.8 | 496.3 | 401.9 | 59.2 | 0.73 | 0.18 | 38.4 |
| 5 | 336.9 | 334.8 | 389.4 | 397.5 | 496.2 | 393.6 | 52.5 | 0.61 | 0.16 | 39.1 |
| 2 | 335.9 | 333.9 | 381.9 | 393.3 | 496.1 | 386.1 | 46.0 | 0.56 | 0.14 | 39.5 |
| 1 | 334.8 | 333.1 | 376.8 | 386.1 | 496.0 | 382.4 | 42.0 | 0.47 | 0.13 | 40.2 |
| 0.5 | 333.8 | 332.8 | 370.9 | 381.6 | 494.2 | 375.8 | 37.0 | 0.41 | 0.11 | 40.6 |

Conditions: samples of liquid loviride quench cooled from 508.15 to 298.15 K and then reheated at different rates to 508.15 K.

## Conclusions

The present study revealed that the anti-HIV agent, loviride, showed temperature dependent stability. This must be taken into account when preparing solid dispersions of this poorly soluble drug using melt extrusion. Overall, this study shows that the applied DSC procedure may serve as a rapid tool to assess the suitability of a drug substance to be processed by hot melt extrusion.

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