A new approach for the estimation of the melting enthalpy of metastable crystalline compounds using differential scanning calorimetry

Application to the two crystallographic forms of Etiracetam

**Christelle Herman · Tom Leyssens ·
Valérie Vermylen · Véronique Halloin ·
Benoît Haut**

Abstract This article focuses on the development of an innovative method, based on thermodynamic considerations and with the use of Differential Scanning Calorimetry (DSC), for the estimation of the melting enthalpy of crystalline compounds which are metastable near their melting temperature. The curves obtained, at various heating rates, are analysed in two steps. In the first step, the area of a zone generated by the melting endothermic peak is calculated following a specific method. In the second step, the melting enthalpy is extracted from this area through an enthalpy balance. This method is applied to both identified crystallographic forms, named form I and form II, respectively, of Etiracetam (UCB Pharma). The results show that the melting enthalpy of the stable form II compare well with the ones obtained using conventional methods. The curves of the metastable form I present thermal instabilities (partial solid–solid polymorphic transition and beta-recrystallization) near the form I melting peak leading to difficulties for a direct determination of the melting enthalpy by conventional methods. The proposed method is therefore very useful for the estimation of the form I melting enthalpy.

Keywords Differential scanning calorimetry ·
Melting enthalpy · Enthalpy balance · Thermodynamic ·
Etiracetam

C. Herman (✉) · V. Halloin · B. Haut
Transfers, Interfaces and Processes Department, Chemical Engineering Unit, Université Libre de Bruxelles, 50 Avenue Franklin D-Roosevelt, CP 165/67, 1050 Brussels, Belgium
e-mail: christelle.herman@ulb.ac.be

T. Leyssens
Institute of Condensed Matter and Nanosciences, Université Catholique de Louvain, 1 Place Louis Pasteur, 1348 Louvain-La-Neuve, Belgium

V. Vermylen
UCB Pharma, 60 Allée de la Recherche, 1070 Braine l'Alleud (Brussels), Belgium

Introduction

Small organic compounds of pharmaceutical interest can often crystallize under different crystallographic solid forms, only one of which is usually stable under given conditions. The development of a large-scale synthesis of the desired crystallographic form starts by a screening allowing detecting the possible crystallographic forms [1]. Solid state properties such as solubility, melting temperature, specific heat capacity, melting enthalpy, XRPD patterns, Raman or IR spectra, etc., are form dependent and can be used to characterize and identify the different crystallographic forms of interest. Determining these properties for the most stable form is usually quite straightforward, but less so when dealing with metastable forms [2].

Differential Scanning Calorimetry (DSC) is commonly used to evaluate most of the thermal properties of solid states, such as melting temperatures and enthalpies or specific heat capacities [3]. Whilst recent contributions focus on methodologies for an accurate and precise determination of the melting temperature and the specific heat capacity, particularly when, for instance, dealing with metastable compounds, few are concerned with the melting enthalpy. However, due to eventual transitions towards the more stable form occurring during the analysis, the

determination of the melting enthalpy of a metastable form can be difficult or impossible using a conventional data treatment. In this article, a new method is therefore developed for the determination of the melting enthalpy of a metastable form using DSC analysis. This method is then applied to both identified forms of Etiracetam (UCB Pharma), a compound that shows two distinct enantiotropically related forms. The method developed was validated by a comparison between the data of the melting enthalpy of the stable form obtained with the proposed method and the data obtained with conventional data treatments. The method was consequently applied to estimate the melting enthalpy of the metastable form of the Etiracetam compound.

Conventional data treatment

The melting enthalpy is defined as the energy per unit mass needed for a solid–liquid phase transition to occur. The determination of the melting enthalpy is commonly obtained using DSC. Most DSC instruments are of a heat flux design [4]. As an outcome of the analysis, the DSC equipment provides DSC curves. The DSC curve is the graphical representation of the difference between the heat flux provided to the crucible containing the sample and the heat flux provided to the reference crucible (ΔW/J s^{-1}) as a function of time (t/s) or as a function of the temperature (T/K). In this article, DSC curve, or curve, is used either for the functions $\Delta W(t)$ or $\Delta W(T)$ than for their graphical representation. Common practise for the determination of the melting enthalpy of a pure compound consists in calculating the area of the endothermic peak corresponding to the melting observed on a curve, which is commonly called the melting peak (Fig. 1) [5]. This is achieved by integrating, between an initial and a final temperature, the difference between the experimental curve and a virtual baseline [6]. The initial temperature (T_i) is chosen some degrees below
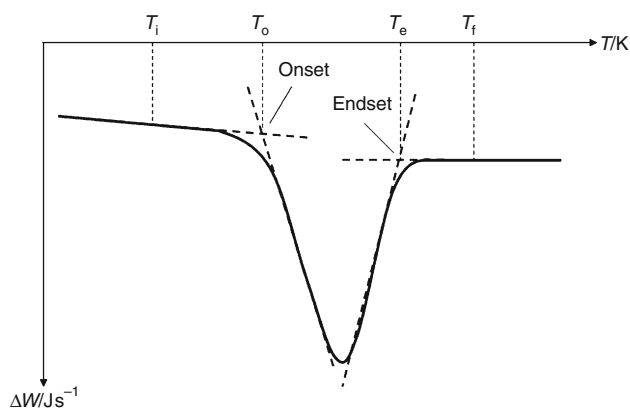
the onset of the melting peak (T_o). At this temperature, the compound is in solid phase (Fig. 1). The final temperature (T_f) is chosen some degrees beyond the endset of the melting peak (T_e). At this temperature, the compound is in liquid phase (Fig. 1). This final temperature has to be chosen lower than the decomposition temperature, if this latter is present, to avoid interference of other thermal events. It can be noted that, using the conventional presentation of the curves, the melting peak is a 'negative peak', even if the melting is an endothermic event.

Two virtual baselines are commonly recommended for the determination of a melting peak, which is not contaminated by any other overlapping effects and for which the specific heat capacities of the solid and the liquid phases are quite different [6, 7]. These two virtual baselines are presented by dotted lines in Fig. 2. The first of these baselines, the 'integral tangential' virtual baseline, is tangent to the curve before and after the first order transition peak (Fig. 2a). The second of these baselines, the 'integral
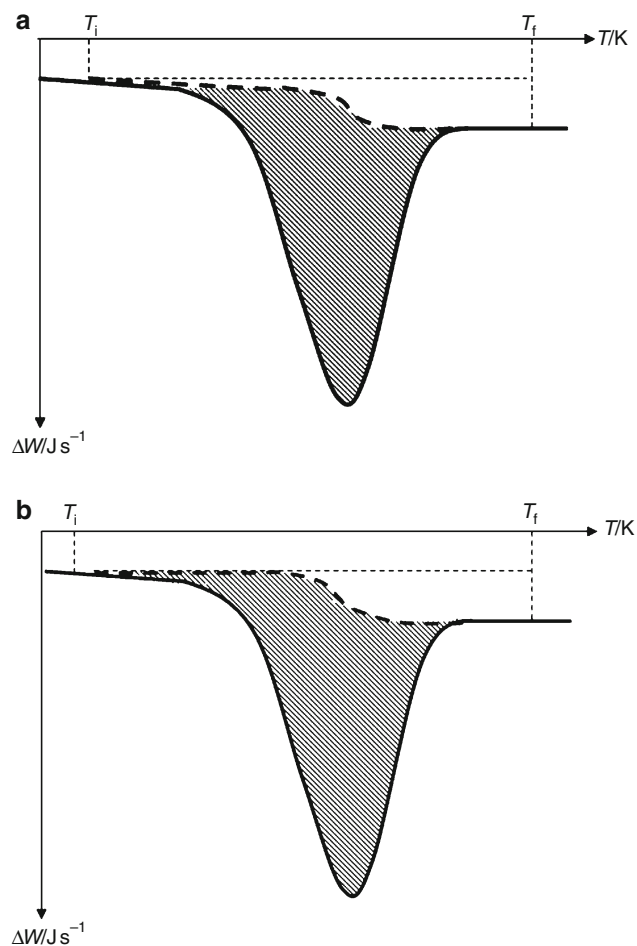

Fig. 2 By *dotted lines* **a** the 'integral tangential' virtual baseline, **b** the 'integral horizontal' virtual baseline (T_i initial temperature and T_f final temperature). The area of the zone highlighted in *grey* is used for the determination of the melting enthalpy (Eq. 1)

Fig. 1 Curve presenting the melting peak of a pure compound (T_i initial temperature, T_o temperature onset, T_e temperature endset and T_f final temperature)

horizontal' virtual baseline, is horizontal at both sides of the transition peak (Fig. 2b).

The melting enthalpy of the pure compound, Δh_m ($J\ g^{-1}$), at its melting temperature, T_m, is given by Eq. 1, where area ($J\ s^{-1}\ K$) is the area of the zone highlighted in grey in Fig. 2a and b, b ($K\ s^{-1}$) is the heating rate and m_s (g) is the sample mass. The accuracy, the precision and the reproducibility of the results involve both the experimental conditions and the experimental device [5, 8, 9]. In particular, the determination of the melting enthalpy is influenced by factors such as the calibration of the equipment, the sample mass, the heating rate, the specific heat capacity change on melting, the virtual baseline, the initial and final temperatures of integration, etc. [10–12]. It is shown in literature that the uncertainty associated with the melting enthalpy value determined with DSC is between 3 and 15% [5].

$$\Delta h_m(T_m) = \text{area}/(b.m_s). \tag{1}$$

Whilst the conventional data treatment for the determination of the melting enthalpy knows a widespread use, especially in the pharmaceutical industry [3, 13, 14], it remains relatively difficult to apply for metastable compounds. Indeed, physical instabilities of the metastable forms may lead to phase transformations during heating before or near its melting temperature and to difficulties in obtaining a pure melting peak. These phenomena are undesirable for melting enthalpy determinations [6]. To the best of our knowledge, no specific studies are encountered in the literature for the estimation of the melting enthalpy of such metastable crystallographic compounds.

Etiracetam compound

Etiracetam is encountered as an intermediate to the synthesis of Leviteracetam, the active ingredient of KEPPRA®, an anti-epileptic drug commercialized by UCB Pharma (Fig. 3). Etiracetam can crystallize in two distinct enantiotropically related crystallographic forms, hereafter called form I and form II. The solid–solid transition temperature of this polymorphic system is equal to 303.65 K: the form I is the stable crystallographic form below 303.65 K, whilst the form II is the stable crystallographic form beyond this temperature [15, 16]. Furthermore thermogravimetric analyses (TGA) show a 0.5% mass loss near

Fig. 3 Molecular structure of the Etiracetam compound
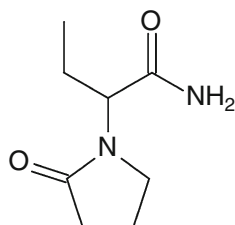
to 419 and 432 K for thermal analyses realized at 5 and 10 K min^{-1}, respectively (SI, Fig. S1).

All the thermal analyses measurements were performed on a METTLER TOLEDO DSC823^e fitted with a 34 places autosampler and a refrigerated chiller unit. The instrument was calibrated for temperature and heat flow accuracies using indium standard. Samples were encapsulated in METTLER TOLEDO standard 40 µL aluminium perforated-lid crucibles which were hermetically sealed after weighing on a METTLER TOLEDO analytical balance. All experiments were performed under a continuous nitrogen flow. The control of the DSC equipment and the analyses of the data were realized using the METTLER TOLEDO STAR^e software. The measurement crucibles were filled with 4 mg of form I or form II crystals. The temperature profile consisted in a sole dynamic segment from an initial to a final temperature without an isothermal segment neither at the initial temperature nor at the final temperature. The temperature domain extended from 303.15 to 403.15 K. Four heating rates were used: 1, 2, 5 and 10 K min^{-1}.

Form II

Curves obtained when heating form II crystals at various heating rates (1, 2 and 5 K min^{-1}) are presented in Fig. 4. The only thermal transformation presents on the curves is the melting of the form II at its melting temperature. The melting peak does not show overlapping effect: this indicates that a single crystal form of high purity is, most likely, present. The melting enthalpy can therefore easily be determined using conventional data treatments selecting the 'integral tangential' or the 'integral horizontal' virtual baselines. The areas highlighted in Fig. 4 were obtained by selecting the 'integral tangential' virtual baseline.[1]

Form I

Curves obtained when heating form I crystals at various heating rates (1, 2, 5 and 10 K min^{-1}) are presented in Fig. 5. It can be observed that the form I curves present a more complex thermal behaviour than the form II curves (Fig. 4). The observed peaks, related to phenomena that may occur during heating the metastable form I, are numbered from 1 to 4. Upon heating the form I at 1 and 2 K min^{-1}, an endothermic peak appears near to 373.15 K (100 °C). This peak (numbered 1 in Fig. 5) characterizes the solid–solid polymorphic transition from the metastable form I to the stable form II. In agreement with Burger's rules, this peak is endothermic for an enantiotropic

---

[1] DSC figures are given in degree celsius. The program used does not allow the conversion to Kelvin.

Fig. 4 Curves of the form II (1, 2 and 5 K min^{-1}). The area highlighted were obtained by selecting the 'integral tangential' virtual baseline (116 °C = 389.15 K and 125 °C = 398.15 K) (see Footnote 1)
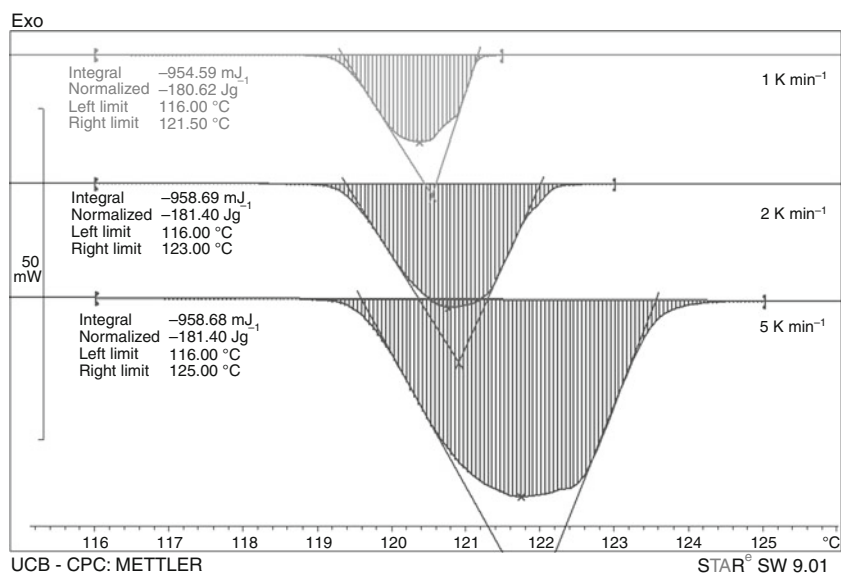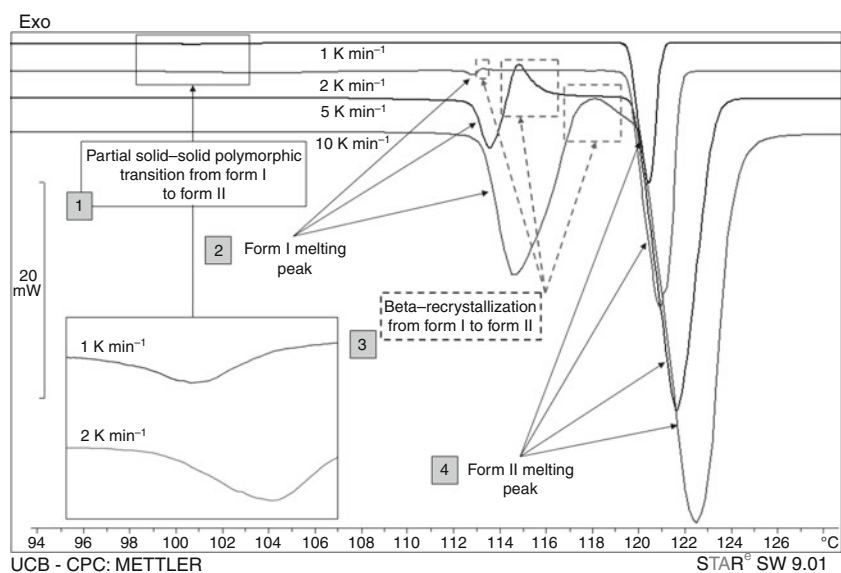

Fig. 5 Curves of the form I presenting several peaks: (1) solid–solid polymorphic transitions from form I to form II, (2) the melting of the form I, (3) beta-recrystallizations from form I to form II and (4) the melting of the form II (94 °C = 367.15 K and 128 °C = 401.15 K) (see Footnote 1)

polymorphic system [17]. Nevertheless, this transition is incomplete as shown by the presence of a small endothermic peak near 385.15 K (112 °C) corresponding to the melting of metastable form I crystals. The solid–solid polymorphic transition is more likely to occur the longer the time period spent above the solid–solid transition temperature is, or, in other words, the smaller the heating rate is [13]. The peak numbered 1 in Fig. 5 can therefore no longer be observed on the curves obtained at 5 and 10 K min^{-1}. At these heating rates, the first peak observed correspond to the melting of the form I (numbered 2 in Fig. 5). The resulting liquid phase successively recrystallizes into stable form II crystals; this phenomenon, called beta-recrystallization, is characterized by the (exothermic) peak numbered 3 in Fig. 5 [13]. The last (endothermic) peak (numbered 4 in Fig. 5) corresponds to the melting of the stable form II crystals. Due to such thermal transformations interfering with the melting of the metastable form, (i) the exact amount of compound undergoing melting is unknown, (ii) the integration domain is difficult to identify and (iii) no ideal virtual baseline can be selected, especially when the curve corresponds to the sum of simultaneously occurring processes [6]. The melting enthalpy of such a metastable compound is therefore quite difficult to determine. Therefore, the objective of the study presented in this article is to develop, using DSC, a new method for the estimation of the melting enthalpy of a crystalline compound, that can be used also when this

compound is metastable before or at its melting temperature. The method was then applied to the Etiracetam compound, as this compound presents a metastable crystallographic form whose determination of its melting enthalpy at its melting temperature is impossible using conventional data treatments.

Thermodynamically based method

The proposed method for the determination of the melting enthalpy is detailed below and can be summarized as follows. First, the area of a well-specified zone, related to the melting peak of the compound, is measured on the curve of this compound. Second, the melting enthalpy is extracted from this area using an enthalpy balance.

Specified zone related to the melting peak

Stable compound—form II

The zone considered on the curve of a stable compound for the determination of its melting enthalpy is presented in Fig. 6a. This zone is limited to the left by the temperature T_i and to the right by the temperature T_f. T_i has to be chosen a few degrees below the onset of the melting peak (T_o); at this temperature, the sample is in solid phase. T_f has to be chosen some degrees above the endset of the melting peak (T_e) and below the decomposition temperature: at this temperature, the sample is in liquid phase. The bottom limit of the zone is the experimental curve ($f_{DSC}(T)$ in J s^{-1}). The upper limit of the zone is a 'horizontal left' virtual baseline ($f_{base}(T)$ in J s^{-1}). This later represents the curve that would be obtained when heating the solid sample from T_i to T_f under the hypothesis that this solid does not undergo a transformation and assuming that its specific heat capacity (c_p in J g^{-1} K^{-1}) remains constant (equal to $c_p(T_i)$).

Metastable compound—form I

The curve of metastable compounds often presents complex phenomena interfering with the melting peak. Based on Fig. 5, the peaks that may be observed on a curve whilst heating a metastable compound are schematically presented in Fig. 7a and numbered from 1 to 4. These peaks, respectively, correspond to: (1) the partial solid–solid polymorphic transition form metastable to stable crystals, (2) the metastable crystals melting, (3) the beta-recrystallization in liquid phase leading to the formation of stable crystals and (4) the stable crystals melting. The zone considered on the curve of a metastable compound for the determination of its melting enthalpy is highlighted in grey
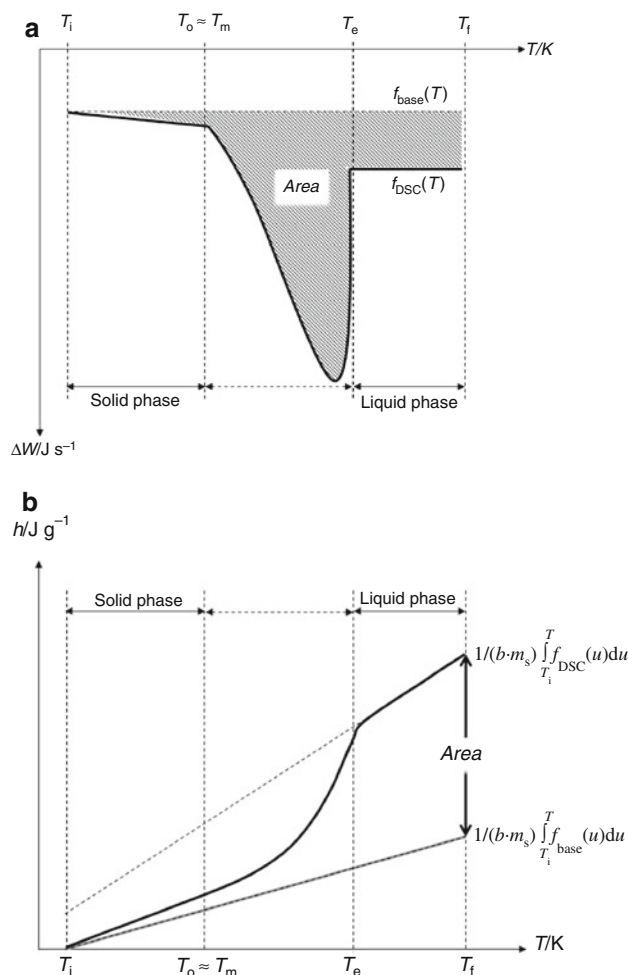

Fig. 6 **a** DSC curve of a stable compound where $f_{base}(T)$ refers to the 'horizontal left' virtual baseline and $f_{DSC}(T)$ refers to the experimental curve. **b** Integration of $f_{base}(T)$ and $f_{DSC}(T)$ (T_i initial temperature, T_m melting temperature, T_o temperature onset, T_e temperature endset, T_f final temperature, b heating rate and m_s sample mass)

in Fig. 7a. It is expected that similar curves would be obtained for other metastable crystalline compounds but, as every compound presents its particularities, it may be possible that an adaptation of the specified zone considered would be required. The zone highlighted in grey in Fig. 7a is limited to the left by the temperature T_i and to the right by the temperature T_f. At the temperature T_i, the metastable compound is in solid phase and not (partially) transformed into the stable compound. At the temperature T_f, the sample is in liquid phase and has not yet decomposed. Therefore, the thermal events that might interfere with the melting peak (partial solid–solid polymorphic transition, beta-recrystallization…) occur at temperatures between T_i and T_f. As previously, $f_{DSC}(T)$ is the experimental curve and $f_{base}(T)$ is the 'horizontal left' virtual baseline at the initial temperature. Parts of the zone highlighted in grey in

Fig. 7 **a** DSC curve of a metastable compound where $f_{base}(T)$ refers to the 'horizontal left' virtual baseline and $f_{DSC}(T)$ refers to the experimental curve. The numbers from 1 to 4 refer to the four possible peaks appearing in the experimental curve. **b** Integration of $f_{base}(T)$ and $f_{DSC}(T)$ (T_i initial temperature, T_m melting temperature of the metastable compound, T_e temperature endset, T_f final temperature, b heating rate and m_s sample mass)
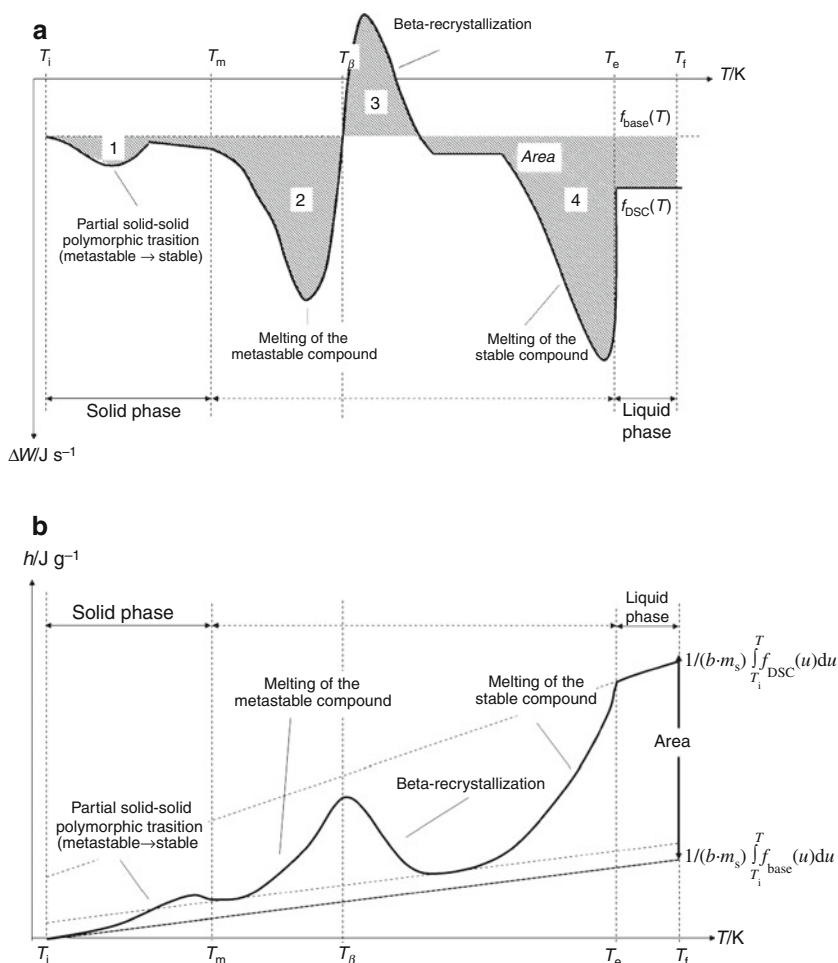

Fig. 7a where $f_{DSC}(T) > f_{base}(T)$ contribute positively to the area and parts of the zone where $f_{DSC}(T) < f_{base}(T)$ contribute negatively to the area.

Extraction of the melting enthalpy from the area of the specified zone

The area of the zone highlighted in Figs. 6a and 7a (area) can be expressed by Eq. 2 where u is the integration variable of the temperature T (K). The graphical representations of the function associated to the two terms of Eq. 2 (integration of $f_{DSC}(T)$ and $f_{base}(T)$) are presented in Figs. 6b and 7b, respectively, corresponding to the Figs. 6a and 7a. The first term in Eq. 2 (J g^{-1}) is, per unit mass, the enthalpy variation of the sample when its temperature is increased from T_i to T_f. It can be observed in Figs. 6b and 7b that the enthalpy pathway from T_i to T_f can be complex. However, as the enthalpy is a state function, this final enthalpy variation is independent of how the transformation is realized between T_i and T_f. Therefore, this final enthalpy variation can be written as being the sum of the

three following components: (i) the energy required to heat the solid from T_i to its thermodynamic melting temperature (T_m), (ii) the melting enthalpy at the thermodynamic melting temperature and (iii) the energy required to heat the resulting liquid from T_m to T_f (Eq. 3). Considering the definition of $f_{base}(T)$, Eq. 4 is straightforward. Using Eqs. 3 and 4, Eq. 2 can therefore be transformed into Eq. 5. The melting enthalpy can then be extracted from Eq. 5 to obtain Eq. 6. In the proposed method, Eq. 6 is used to calculate the melting enthalpy at the thermodynamic melting temperature, $\Delta h_m(T_m)$, from the measurement of the area of the specified zone, area. The evaluation of the melting enthalpy of a compound with the developed method requires the knowledge of its thermodynamic melting temperature, T_m, and the knowledge of the specific heat capacities of its solid and liquid phases, $c_{p,s}$ and $c_{p,l}$, respectively.

$$area/(b \cdot m_s) = 1/(b \cdot m_s)\left[\int_{T_i}^{T_f} f_{DSC}(u)du - \int_{T_i}^{T_f} f_{base}(u)du\right]$$

(2)

$$1/(b \cdot m_{\mathrm{s}}) \int_{T_{\mathrm{i}}}^{T_{\mathrm{f}}} f_{\mathrm{DSC}}(u)\mathrm{d}u = \int_{T_{\mathrm{i}}}^{T_{\mathrm{m}}} c_{p,\mathrm{s}}(u)\mathrm{d}u + \Delta h_{\mathrm{m}}(T_{\mathrm{m}})$$
$$+ \int_{T_{\mathrm{m}}}^{T_{\mathrm{f}}} c_{p,\mathrm{l}}(u)\mathrm{d}u \qquad (3)$$

$$1/(b \cdot m_{\mathrm{s}}) \int_{T_{\mathrm{i}}}^{T_{\mathrm{f}}} f_{\mathrm{base}}(u)\mathrm{d}u = c_{p,\mathrm{s}}(T_{\mathrm{i}})(T_{\mathrm{f}} - T_{\mathrm{i}}) \qquad (4)$$

$$\mathrm{area}/(b \cdot m_{\mathrm{s}}) = \int_{T_{\mathrm{i}}}^{T_{\mathrm{m}}} c_{p,\mathrm{s}}(u)\mathrm{d}u + \Delta h_{\mathrm{m}}(T_{\mathrm{m}})$$
$$+ \int_{T_{\mathrm{m}}}^{T_{\mathrm{f}}} c_{p,\mathrm{l}}(u)\mathrm{d}u - c_{p,\mathrm{s}}(T_{\mathrm{i}})(T_{\mathrm{f}} - T_{\mathrm{i}}) \qquad (5)$$

$$\Delta h_{\mathrm{m}}(T_{\mathrm{m}}) = \mathrm{area}/(b \cdot m_{\mathrm{s}}) - \int_{T_{\mathrm{i}}}^{T_{\mathrm{m}}} c_{p,\mathrm{s}}(u)\mathrm{d}u$$
$$- \int_{T_{\mathrm{m}}}^{T_{\mathrm{f}}} c_{p,\mathrm{l}}(u)\mathrm{d}u + c_{p,\mathrm{s}}(T_{\mathrm{i}})(T_{\mathrm{f}} - T_{\mathrm{i}}). \qquad (6)$$

Results and discussion

The method was first validated by a comparison between the melting enthalpies of the stable form II obtained with the thermodynamically based method and with the conventional data treatment. Then, the method is used for the estimation of the melting enthalpy of the form I Etiracetam metastable compound, a compound for which the use of the conventional data treatment does not allow to determine a melting enthalpy. Before the determination of the melting enthalpies with the thermodynamically method, the melting temperature and the specific heat capacities of the solid phase (form I crystals and form II crystals) and the liquid phase were determined using DSC. The experimental methods and results are given in the Supporting information.

Validation of the method: determination of the stable form II melting enthalpy

For each heating rate, various initial and final temperatures were used to test the robustness of the developed method (Eq. 6). Nine different initial temperatures (T_{i}) were considered: 387.15, 387.65, 388.15, 388.65, 389.15, 389.65, 390.15, 390.65 and 391.15 K. Even if the thermodynamic form II melting temperature is found to be 392.39 K, all curves showed deviation from the 'horizontal left' virtual baseline at approximately 391.15 K, and therefore no

temperature was considered beyond. As it can be observed in Fig. 4, for a given sample mass, the width of the melting peak increases with the heating rate. This is partially due to the thermal inertia of the sample crucible [8, 11, 12]. The integration domain has therefore to cover a wider temperature range for the highest heating rates than for the smallest ones. The lowest final temperature (T_{f}) considered was 394.65, 396.15, 398.65 and 402.15 K, for the curves obtained at 1, 2, 5 and 10 K min^{-1}, respectively. Five other final temperatures were considered at each heating rate. They were obtained by successive increase of 0.5 K, starting from the lowest final temperature.[2] The decomposition temperature was at least 10 K above all of the final temperatures considered: no interference due to mass loss or decomposition was therefore expected. As the specific heat capacities before and after the melting are quite different, the use of the 'integral tangential' or the 'integral horizontal' virtual baselines is recommended when using the conventional methods [6, 7]. These two conventional methods are hereafter called as conventional method 'Int. Hor.' and conventional method 'Int. Tang.'.

The melting enthalpies extracted from the curves obtained when heating the form II at 1 and 5 K min^{-1} are presented in Figs. 8 and 9, respectively. In these figures, the form II melting enthalpy (y-axis) obtained by the method proposed in this study (Met.) is compared with the results obtained by the conventional method 'Int. Hor.' and 'Int. Tang.'. The influence of the choice of the initial temperature (x-axis) is presented in Figs. 8a and 9a for the lowest final temperatures: 394.65 K for 1 K min^{-1} and 398.65 K for 5 K min^{-1}, respectively (in legend). The influence of the choice of the final temperature (x-axis) is presented in Figs. 8b and 9b for the largest initial temperature (391.15 K) for 1 and 5 K min^{-1}, respectively (in legend).

Even if the method developed shows a higher melting enthalpy compared to the conventional methods, Figs. 8 and 9 show that the experimental results obtained are in good agreement with the ones obtained using the conventional methods. The relative difference is about 0.5% when comparing the melting enthalpy of the form II determined with the method proposed and the values obtained with the conventional method 'Int. Hor.'. This relative difference of 0.5% is comparable to the relative difference between the two conventional methods. It can therefore be concluded that, for the determination of the melting enthalpy of a stable compound, the method gives results comparable to the conventional methods.

---

[2] All initial and final temperature combinations, for each heating rate, are summarised in the Supporting information (SI, Tables S2, S3, S4 and S5).
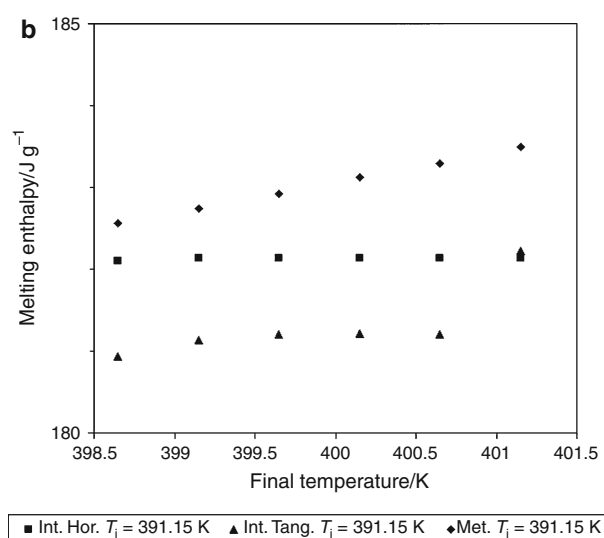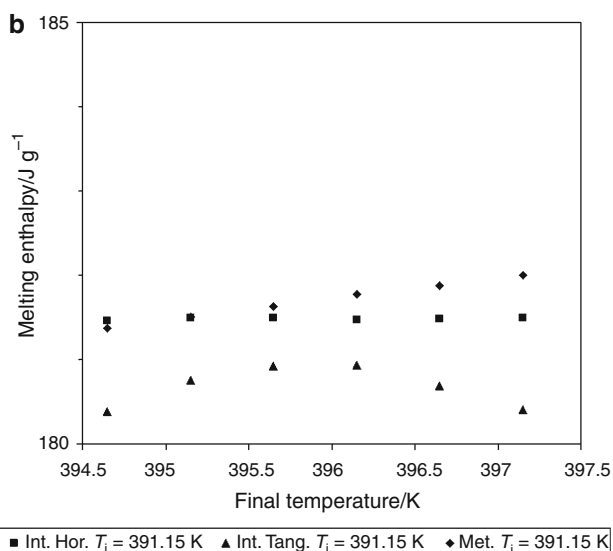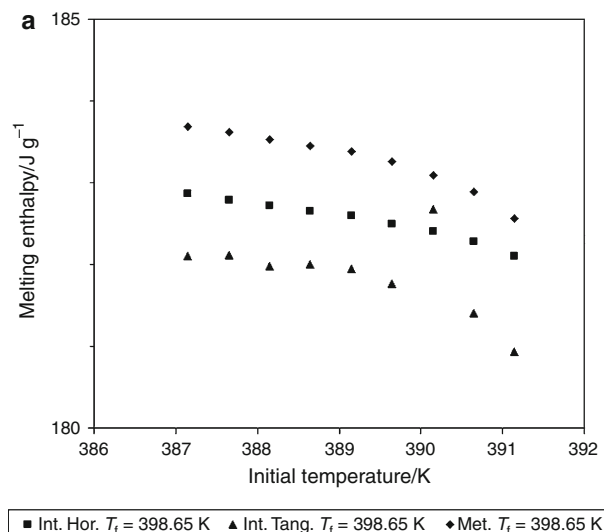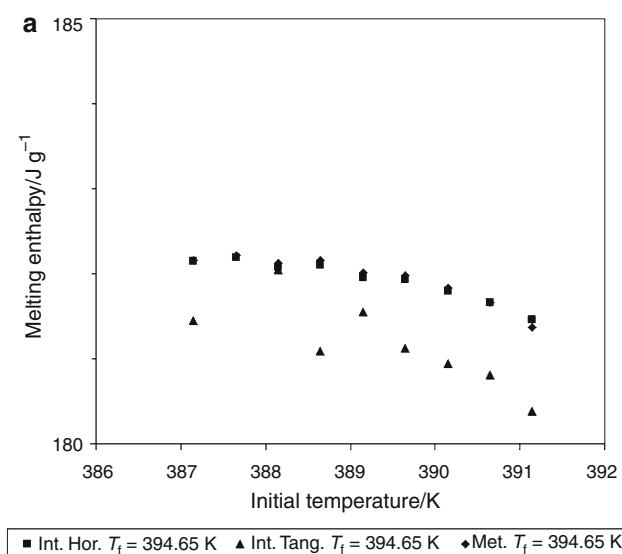
Fig. 8 Melting enthalpy of the form II extracted from the curve obtained at 1 K min^{-1} with the conventional methods 'Int. Hor.' and 'Int. Tang.' and with the method proposed in this study ('Met.'). Influence of the choice of **a** the initial temperature for a final temperature = 394.65 K and **b** the final temperature for an initial temperature = 391.15 K

Fig. 9 Melting enthalpy of the form II extracted from the curve obtained at 5 K min^{-1} with the conventional methods 'Int. Hor.' and 'Int. Tang.' and with the method proposed in this study ('Met.'). Influence of the choice of **a** the initial temperature for a final temperature = 398.65 K and **b** the final temperature for an initial temperature = 391.15 K

As highlighted by Fig. 8a, the results obtained at 1 K min^{-1} using the method proposed compare very well with the results obtained using the conventional 'Int. Hor.' baseline for each initial temperature investigated. As highlighted by Fig. 8b, this comparison is affected by the choice of the final temperature. A stronger resemblance is observed between the two absolute values when choosing a final temperature close to the endset of the melting peak. This can be explained by a larger experimental error on the specific heat capacity of the liquid phase than that of the solid phase. A less good resemblance is obtained when

using a higher heating rate (Fig. 9). This may be explained by an increase of the inertial effect and possible heat losses when using higher heating rates. These errors increase upon an increase of the total temperature range considered, explaining why the difference between both values decrease upon an increase of the initial temperature (Fig. 9a). Similar observations can be made from the curves obtained at 2 and 10 K min^{-1}.[3]

---

[3] These results are given in the Supporting information (SI, Figs. S5 and S6).

Table 1 Comparison of the form II melting enthalpy extracted from the curves obtained at different heating rates with the conventional method 'Int. Hor.' and 'Int. Tang.' and with the method proposed in this study ('Met.') in the optimal temperature domain

b/K min^{-1}	T_i/K	T_f/K	$\Delta h_m(T_m)$/J g^{-1}		
			Int. Hor.	Int. Tang.	Met.
1	391.15	394.65	181.46	180.38	181.37
2	391.15	396.15	181.91	180.99	181.94
5	391.15	398.65	182.10	180.93	182.56
10	391.15	402.15	182.16	180.32	183.06

T_i initial temperature, T_f final temperature

Based on the observations mentioned above, the better conditions for an accurate determination of the form II melting enthalpy with the method proposed are to use (i) the smallest heating rates possible (however larger enough to avoid a lack of sensibility of the DSC equipment), (ii) an initial temperature as high as possible to limit the effect due to the imprecision of the specific heat capacity of the solid phase and (iii) a final temperature as low as possible to reduce the errors due to imprecision of the specific heat capacity of the liquid phase. Ideal temperature ranges are therefore dependent on the heating range used.

The results of the form II melting enthalpy, choosing best possible initial and final temperatures, are reported in Table 1. The table compares the results obtained at different heating rates using the conventional methods and the method presented here. The results obtained using the conventional method 'Int. Tang.' are almost independent of the heating rate. In contrast, the results obtained using the conventional method 'Int. Hor.' and the method proposed in this study show a slight dependence on the heating rate. However, it should be noted that the melting enthalpy obtained using the method presented here varies by a mere 1% upon a tenfold increase of the heating rate.

To study the sensitivity of Eq. 6 to the precision of the thermodynamic data, a sensitivity study was realized by determining the enthalpy that would be predicted if the melting temperature and the specific heat capacity of the form II were increased by 0.01 and 3%, respectively, as these were the experimental errors determined for these thermodynamic parameters in the context of this study (Supporting information). The influence of these increases on the value of the melting enthalpy extracted using Eq. 6 from the curves obtained at 1 K min^{-1} is presented in Fig. 10. The observed modification of the form II melting enthalpy is a mere 0.5%.

Use of the method: estimation of the metastable form I melting enthalpy

The form I curve exhibits partial solid–solid polymorphic transitions and beta-recrystallizations towards the form II,
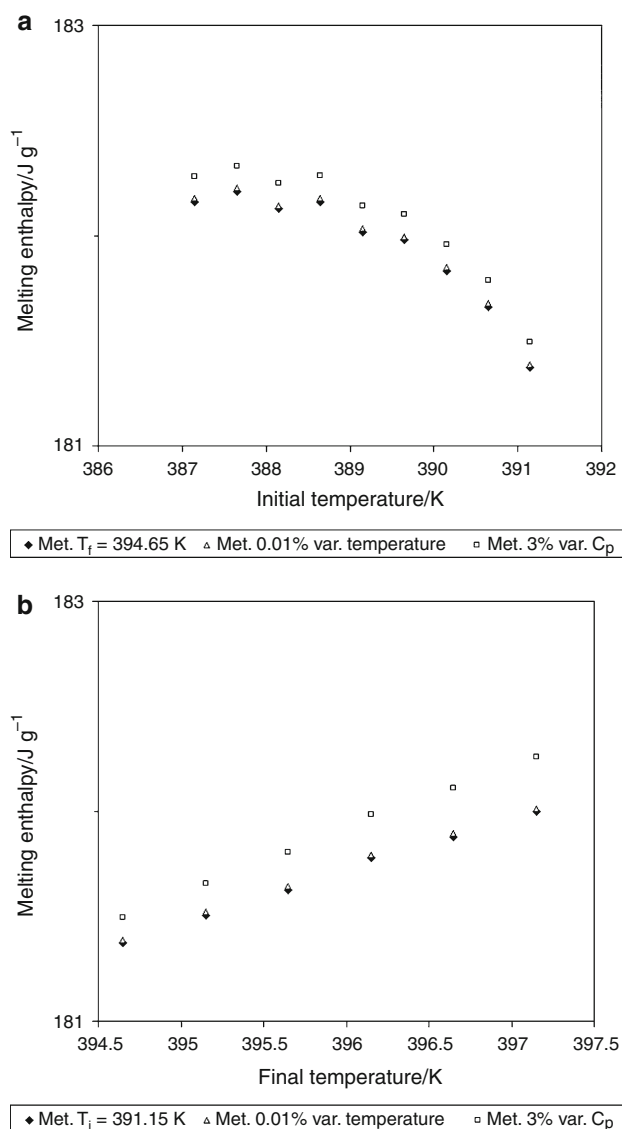
Fig. 10 Sensitivity study of Eq. 6 to a 0.01% variation of the form II melting temperature and a 3% variation of the form II specific heat capacity using the curve obtained at 1 K min^{-1}. Met. refers to the results presented in Fig. 8. Influence of the choice of **a** the initial temperature for a final temperature = 394.65 K and **b** the final temperature for an initial temperature = 391.15 K

interfering with the melting of the form I (Fig. 5). This, amongst others, inhibits a direct measurement of the form I melting enthalpy. However, the method presented in this article circumvents these issues by considering a larger integration domain, including all thermal events between the solid and the liquid phases (Fig. 7a and b). The initial and final temperatures thus have to be chosen with care. In these conditions, the total area integrated can always be expressed by Eq. 6. The highest initial temperatures (T_i) depend on the heating rate at which the curves were obtained. For technical reasons, the curve obtained when heating the form I at 1 K min^{-1} could not be used. For the

curve obtained at 2 K min⁻¹, the highest initial temperature was chosen below the onset of the partial solid–solid polymorphic transition peak: 369.15 K. For the curves obtained at 5 and 10 K min⁻¹, the highest initial temperature was chosen below the form I melting peak: 376.15 and 380.15 K, respectively. Indeed, as none of the curves at these heating rates showed a polymorphic solid–solid transition endothermic event, the hypothesis that crystals remain in the form I crystallographic form until the form I melting temperature was assumed. Lower initial temperatures were considered at each heating rate. They were obtained by successive decrease of 0.5 K, starting from the highest initial temperature. For each curve, several final temperatures, beyond the endset of the form II melting peak and below the decomposition temperature, were considered. The lowest final temperature was 396.15, 399.15 and 401.65 K, for the curves obtained at 2, 5 and 10 K min⁻¹, respectively. Two other final temperatures were considered at each heating rate. They were obtained by successive increase of 0.5 K, starting from the lowest final temperature.[4]

The melting enthalpy of the form I extracted from the curves obtained when heating the form I at 2, 5 and 10 K min⁻¹ are presented in Fig. 11a, b and c, respectively. The form I melting enthalpy was estimated by the method presented in this study. To better compare the different results amongst each other the y- and x-axis scales of the different sub-figures of Fig. 11 are the same.[5] The y-axis presents the melting enthalpy and extends from 180 to 188 J g⁻¹. The x-axis, presenting the initial temperature of the integration domain on the curve, extends from 365 to 385 K. The temperature range in which the solid–solid polymorphic transition peak is observed on the curve obtained at 2 K min⁻¹ (Fig. 5) is highlighted in grey in Fig. 11a. The form I melting temperature is indicated in all sub-figures of Fig. 11. All chosen initial temperatures lie at least 5 °C below the thermodynamic form I melting temperature. The final temperature of the integration domain on the curve (T_f) is given in the legend.

Considering each sub-figure separately, it can be noted that the estimation of the form I melting enthalpy does not vary significantly with the final temperature of integration. However, it is shown in Fig. 11 that the results vary significantly from a heating rate to another. The discrepancy of the values obtained for the three heating rates investigated can be due to the difference of the temperature
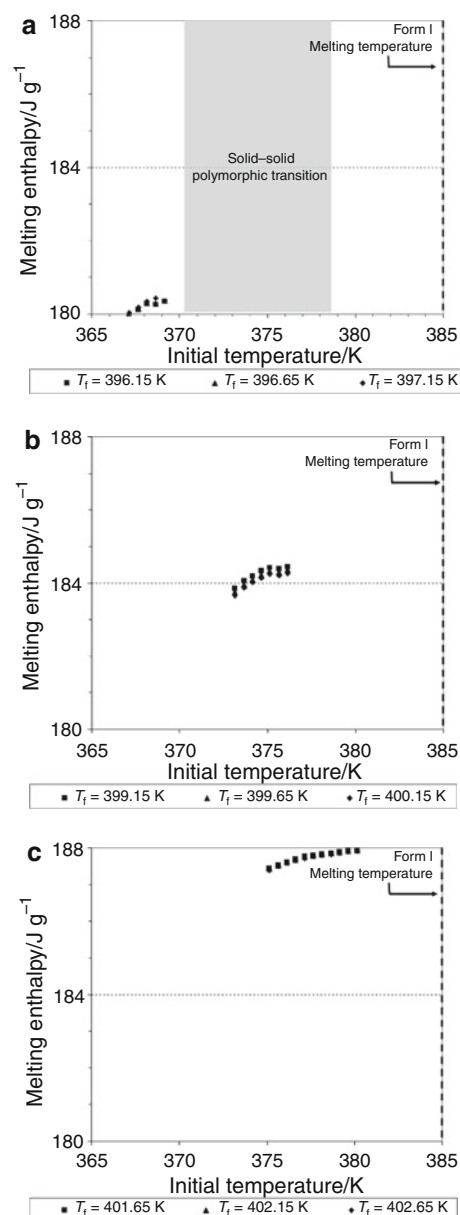

Fig. 11 Melting enthalpy of the form I extracted from the curve obtained at **a** 2 K min⁻¹, **b** 5 K min⁻¹ and **c** 10 K min⁻¹ with the method presented in this study. Influence of the choice of the initial temperature for three final temperatures

ranges of integration considered in the Eq. 6. Smaller the heating rate is, smaller the highest initial temperature that can be considered is. The specific heat capacity of the form I crystals has therefore to be considered in a wide temperature range. In contrast, higher the heating rate is, higher the smallest final temperature that can be considered is. The specific heat capacity of the liquid phase has therefore to be considered in a wide temperature range. In both case, this implies increased errors in the estimation of the form I melting enthalpy. Finally, the increase of the form I melting enthalpy observed when increasing the

---

[4] All initial and final temperatures combination, for each of the heating rates, are summarised in the Supporting information (SI, Tables S6, S7 and S8).

[5] A zoom of each sub-figure of Fig. 11 is given in the Supporting information (SI, Fig. S7).

Table 2 Comparison of the form II and form I melting enthalpies extracted from the curves obtained at different heating rates with the method proposed in this study ('Met.') in the optimal temperature domain

b/K min^{-1}	Form II			Form I		
	T_i/K	T_f/K	$\Delta h_m(T_m)$/ J g^{-1}	T_i/K	T_f/K	$\Delta h_m(T_m)$/ J g^{-1}
1	391.15	394.65	181.37	–	–	–
2	391.15	396.15	181.94	369.15	396.15	180.34
5	391.15	398.65	182.56	376.15	399.15	184.44
10	391.15	402.15	183.06	380.15	401.65	187.91

T_i initial temperature, T_f final temperature

heating rate could also be due to other effects such as the increasing thermal inertia.

Considering that the largest error lies within the estimation of the specific heat capacity, as it was the case for the determination of the stable form II melting enthalpy, the better experimental conditions for the estimation of the metastable form I melting enthalpy are those considering the smallest temperature range of integration. Experimental results extracted from all curves, for the highest initial temperatures and the lowest final temperatures, are summarized in Table 2 for both the form II and the form I. Whilst the variation of the form II melting enthalpy is about 1% upon a tenfold increase of the heating rate, the form I melting enthalpy shows a 4% variation upon a fivefold increase of the heating rate. This variation seems acceptable considering the fact that the method proposed in this study allows estimating the melting enthalpy of a metastable compound that would otherwise be impossible to determine with conventional methods.

Even if an adaptation of the method proposed in this article will be required, it can be expected that this thermodynamically based method can also be applied for the determination of the melting enthalpy of other metastable crystalline compounds for which thermal transformations such as partial solid–solid polymorphic transition or beta-recrystallization interfere with the melting of the metastable form [18].

Conclusions

In this article, a new method for the determination of the melting enthalpy from DSC curves is presented. The method developed is based on a thermodynamic enthalpy balance and requires the knowledge of the melting temperature and the specific heat capacities of the compound in solid and liquid phases. It was shown that, for the stable form II of the Etiracetam compound, the new method proposed for the determination of the melting enthalpy

compares well with the conventional methods using the 'Integral Horizontal' and 'Integral Tangential' virtual baselines. The uncertainty on the results obtained with the method presented in this article is of the same order of magnitude than the uncertainty of the results obtained with the conventional method: 0.5%. The method is, moreover, particularly interesting for the determination of the melting enthalpy of the form I which is metastable below and near its melting temperature. Indeed, due to other thermal events occurring in the temperature range under study, the conventional methods cannot be used. The results highlighted that the value determined for the melting enthalpy was slightly heating rate dependent: 1% variation upon a tenfold increase of the heating rate for the form II and 4% variation upon a fivefold increase of the heating rate for the form I. For this reason, a single value of the melting enthalpy for form I and form II could not be given. Even if an adaptation of the method proposed in this article will be required, it can be expected that this thermodynamically based method can be applied for the determination of the melting enthalpy of other metastable crystalline compounds.

References

1. Mangin D, Puel F, Veesler S. Polymorphism in processes of crystallization in solution—a practical review. Org Process Res Dev. 2009;13:1241–53.
2. Veesler S, Ferte N, Costes MS, Czjzek S, Astier JP. Temperature and pH effect on the polymorphism of Aprotinin (PBTI) in sodium bromide solutions. J Cryst Growth Des. 2004;4(6): 1137–41.
3. Giron D. Thermal analysis and calorimetric methods in the characterisation of polymorphs and solvates. Thermochim Acta. 1995;248:1–59.
4. Boettinger WJ, Kattner UR, Moon KW, Perepezko JH. DTA and heat-flow DSC measurements of alloy melting and freezing, NIST Recommended Practice Guide. 2006.
5. Kolbe E, Wilson LA, Hartel R. A round robin evaluation of differential scanning calorimetry to measure transition enthalpy and temperatures. J Food Eng. 1999;40:95–9.
6. Riesen R. Thermal Analysis Information for Users METTLER TOLEDO: Choosing the right baseline, User Com 25. 2007.
7. Wunderlich B. Thermal analysis. New York: Academic Press; 1990. p. 277–80.
8. Greco A, Maffezzoli A. Correction of melting peaks of different PE grades accounting for heat transfer in DSC samples. Polym Test. 2008;27(1):61–74.
9. Herman, C; PhD Thesis, Université Libre de Bruxelles, Belgium. 2010. Contribution à l'étude de la cristallisation, par refroidissement en cuve agitée, de substances d'intérêt pharmaceutique présentant un polymorphisme cristallin. http://theses.ulb.ac.be/ETD-db/collection/available/ULBetd-12082009-155716/ (2010).

10. Brown ME. Handbook of thermal analysis and calorimetry. Amsterdam: Elsevier; 1998.
11. Marini A, Bernenni V, Flor G, Massarotti V, Riccardi R. An analysis of the factor affecting the peak shape and the quantitative reliability of a heat flux DSC cell. Thermochim Acta. 1985;95(2): 419–24.
12. Marini A, Berbenni V, Massarotti V, Flor G. On the quantitative reliability on heat flux DSC. J Therm Anal Calorim. 1988;33(1): 337–42.
13. Grooff D, Villiers MM, Liebenberg W. Thermal methods for evaluating polymorphic transitions in Nefedipine. Thermochim Acta. 2007;454:33–42.
14. Lara-Ochoa F, Pérez GE, Mijangos-Santiago F. Calorimetric determinations and theoretical calculations of polymorphs of thalidomide. J Mol Struct. 2007;840(1–3):97–106.
15. Herman C, Leyssens T, Vermylen V, Halloin V, Haut B. Towards an accurate and precise determination of the solid–solid transition temperature of enantiotropic systems. J Chem Thermodyn. 2011; 43:677–82.
16. Herman C, Haut B, Halloin V, Vermylen V, Leyssens T. Towards the determination of the solubilities of the two enantiotropically-related crystallographic forms of Etiracetam in methanol. Org Process Res Dev. 2011 (accepted).
17. Burger A, Ramberger R. On the polymorphism of pharmaceutical and other molecular crystals-I. Theory of thermodynamics rules. Mikrochim Acta. 1979;2:259–71.
18. Drebushchak VA, Drebushchak TN, Chukanov NV, Boldyreva EV. Transitions among five polymorphs of chlorpropamide near the melting point. J Therm Anal Calorim. 2008;93(2):343–51.