



Short communication

## Determination of the nateglinide polymorphic purity through DSC

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## ABSTRACT

It is well known that the control of the crystallization of drugs to ensure that only the approved and desired polymorph is present in the formulation is a crucial point of a preformulation study. In this regard, the aim of the present work is to devise a method for the quantification of the polymorphic purity of nateglinide in mixtures formed by polymorphs H and B. In order to achieve this goal, binary systems of known composition have been prepared and the melting peaks of both polymorphs have been recorded by differential scanning calorimetry. Experiments have determined that the method of preparation of the mixtures has to be carefully evaluated. Indeed it has been shown that grinding the samples induces transition from B to H form. Furthermore, it could be observed that the enrichment of the binary mixture with H form is caused by heating. Therefore, after having prepared the mixture without grinding stage, we propose a method to evaluate the content of H polymorph in mixture with the B one from the melting peak of B.

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### 1. Introduction

This paper is the completion of an in-depth physico-chemical study of nateglinide [1,2] in that it constitutes an answer to a common need in the pharmaceutical field to widen the knowledge on the polymorphism [3–6]. The nateglinide, (–)-N-[(trans-4-isopropylcyclohexane carbonyl)-D-phenylalanine], is an oral antidiabetic drug [7] to which recently the industry has paid particular attention. In our previous studies [1,2] we have defined the relationships of relative stability among the three known nateglinide polymorphs (H, B and S) and we have found and characterized new solid modifications of this drug.

Polymorphism is an important topic in the quality control of pharmaceutical products. It is well known that the control of the crystallization of drugs to ensure that only the approved and desired polymorph is present in the formulation is a crucial point of a preformulation study. As a matter of fact, the regulatory authorities underline the importance to limit the polymorphic impurities in the pharmaceutical materials. Since different polymorphs of the same active principle exhibit different physical properties, chemical stability, solubility and dissolution rate, the presence of a different solid form may have an adverse impact on the manufacturing and on the performance of the pharmaceutical product. The partial and unexpected formation of the alternative polymorph could occur during the crystallization process, for example as a con-

sequence of an accidental temperature change, or be induced by mechanical treatments used to prepare the dosage form. The aim of the present work is to build a calibration line for the assessment of the polymorphic purity of nateglinide in binary mixtures formed by polymorphs H and B. In order to achieve this goal, binary systems of known composition have been prepared and the melting enthalpy has been determined by differential scanning calorimetry (DSC).

### 2. Materials and methods

#### 2.1. Sample preparation

Nateglinide polymorphs B and H came from R&D samples (AMSA S.p.A., Como, Italy).

Measurements have been performed both on the pure components and on binary systems prepared according to three different methods:

- 1) *Ground mixtures*: original H and B samples have been weighted on a Mettler analytical balance to an accuracy of 0.01 mg and then manually and gently mixed together in an agate mortar in order to obtain a homogeneous system. The compositions of these mixtures were H:B 20:80 and 80:20 (w:w).
- 2) *Physical mixtures*: original H and B samples first have been separately ground in a mortar in order to break the particles blocks; then, right amounts of the ground pure components have been weighted and mixed in turbula (Willy A. Bachofen) for 20 min, according to the following ratios: H:B 5:95, 10:90, 20:80, 40:60, 60:40 e 20:80 (w:w).

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**Table 1**Onset temperatures ( $T_B$ ,  $T_H$ ), measured ( $\Delta H_{B,mea}$ ,  $\Delta H_{H,mea}$ ) and expected melting enthalpies on the basis of the ground mixtures compositions ( $\Delta H_{B,ex}$ ,  $\Delta H_{H,ex}$ ).

Composition	$T_B$ (°C)	$\Delta H_{B,mea}$ (J/g)	$\Delta H_{B,ex}$ (J/g)	$T_H$ (°C)	$\Delta H_{H,mea}$ (J/g)	$\Delta H_{H,ex}$ (J/g)
H:B=20:80	128.4 ± 0.3	56.3 ± 3.4	71.7	138.1 ± 1.7	42.4 ± 5.4	19.2
H:B=80:20	127.9 ± 0.5	8.7 ± 3.1	17.9	137.9 ± 0.8	88.2 ± 2.9	77.0

**Table 2**Onset temperatures ( $T_B$ ,  $T_H$ ), measured ( $\Delta H_{B,mea}$ ,  $\Delta H_{H,mea}$ ) and expected melting enthalpies on the basis of the mixtures compositions ( $\Delta H_{B,ex}$ ,  $\Delta H_{H,ex}$ ).

Composition	$T_B$ (°C)	$\Delta H_{B,mea}$ (J/g)	$\Delta H_{B,ex}$ (J/g)	$T_H$ (°C)	$\Delta H_{H,mea}$ (J/g)	$\Delta H_{H,ex}$ (J/g)
H:B=5:95	128.7 ± 0.1	79.0 ± 5.7	85.1	138.2 ± 0.1	13.9 ± 7.2	4.8
H:B=10:90	128.3 ± 0.0	77.5 ± 0.4	80.6	138.4 ± 0.1	15.1 ± 0.7	9.6
H:B=20:80	128.1 ± 0.1	70.0 ± 3.1	71.7	138.3 ± 0.1	25.3 ± 3.6	19.2
H:B=40:60	128.8 ± 0.1	56.2 ± 3.1	53.8	138.8 ± 0.2	34.7 ± 3.4	38.5
H:B=60:40	128.8 ± 0.1	35.6 ± 5.4	35.8	139.7 ± 0.0	55.5 ± 5.1	57.7
H:B=80:20	128.3 ± 0.1	13.2 ± 3.4	17.9	138.7 ± 0.3	81.3 ± 3.2	77.0

3) *Binary systems directly prepared in the DSC pan*: the pure H and B samples have been directly weighted in the DSC pans for the analyses in order to obtain samples with percentages of the two forms covering the full composition range.

## 2.2. Apparatus and experimental parameters

Thermal analyses were carried out with a DSC Q2000 apparatus interfaced with a TA 5000 data station (TA Instruments, USA). The DSC instrument was calibrated using ultrapure (99.999%) indium (m.p. = 156.6 °C;  $\Delta H = 28.54$  J/g) as standard. The calorimetric measurements were conducted with sealed standard aluminum pans under a flow of nitrogen (3 L/h) at different heating rate ( $\beta$ , 10–30 K/min). All the enthalpy values reported in this paper are an average of three or more experiments except for the data relating to the binary systems directly prepared in the DSC pan.

## 3. Results and discussion

### 3.1. Ground mixtures

The thermal behaviour of the pure polymorphs B and H used in this work is very similar to that described for the lots studied in our previous work [1]. In particular, the B form melts at  $128.9 \pm 0.3$  °C with enthalpy change of  $89.6 \pm 3.6$  J/g, while the H form melts at  $137.9 \pm 0.4$  °C with enthalpy change of  $96.2 \pm 3.0$  J/g.

The DSC curves of ground mixtures show the melting peaks of both polymorphs. However, the separation extent of these peaks considerably changes with modifying heating rate and system composition. With the composition H:B 20:80 the measurements have been performed at 10 K/min (Fig. 1) because, at higher  $\beta$ , the peaks are partially superimposed, while, with low  $\beta$ , they are separated by a small exothermal effect that is due to the crystallisation of the S polymorph, as described by Bruni [1]. With the composition 80:20, on the contrary, the best heating rate to obtain a good separation between the melting peaks proved to be 30 K/min (Fig. 2).

In Table 1 the onset temperatures ( $T_B$ ,  $T_H$ ) and the measured ( $\Delta H_{B,mea}$ ,  $\Delta H_{H,mea}$ ) enthalpy changes of the two melting peaks are reported together with the expected values on the basis of the mixtures composition ( $\Delta H_{B,ex}$ ,  $\Delta H_{H,ex}$ ). The last have been calculated using the melting enthalpies here measured for the two pure solid forms, B and H. From Table 1 it is clearly evident that the experimental values are significantly different from the expected ones. In particular, for both compositions, the enthalpy change measured for the B form melting is lower than the expected value, while the enthalpy change measured for the H form is higher than the expected one. These experimental evidences indicate that a transition from B polymorph to H polymorph has occurred. Two reasons can be at the origin of this transition: (a) the milling of the two

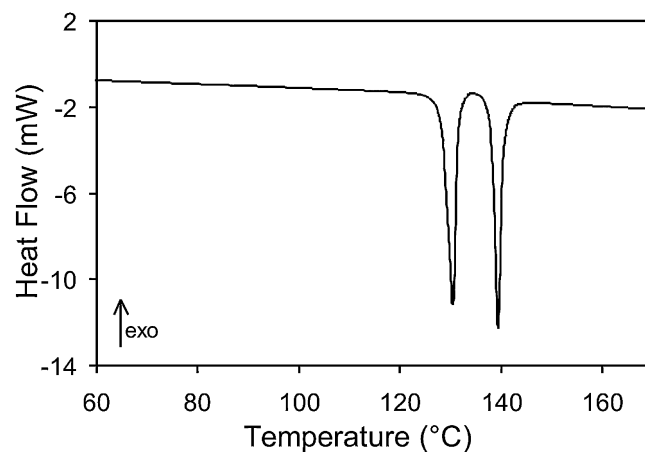


Fig. 1. DSC curve at 10 K/min of a ground mixture with composition H:B=20:80.

components in mortar, although performed without giving great energy; (b) the heating of the mixture during the DSC analysis. In order to identify the true cause, measurements have been carried out on samples prepared through the other two methods described in the experimental section, i.e. with no grinding stage.

### 3.2. Physical mixtures

In the DSC curves of the physical mixtures the melting peaks of both polymorphs are present too and their separation extent again changes with modifying  $\beta$  and system composition. With compositions (H:B) between 5:95 and 40:60, the measurements have been performed at 10 K/min because, with high  $\beta$ , the peaks

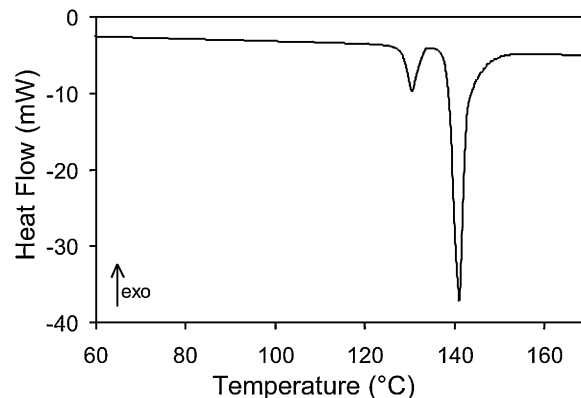


Fig. 2. DSC curve at 30 K/min of a ground mixture with composition H:B=80:20.

**Table 3**  
Onset temperatures ( $T_B$ ,  $T_H$ ), measured ( $\Delta H_{B,mea}$ ,  $\Delta H_{H,mea}$ ) and expected melting enthalpies on the basis of the compositions of the systems directly prepared in DSC pans ( $\Delta H_{B,ex}$ ,  $\Delta H_{H,ex}$ ).

Composition	$T_B$ (°C)	$\Delta H_{B,mea}$ (J/g)	$\Delta H_{B,ex}$ (J/g)	$T_H$ (°C)	$\Delta H_{H,mea}$ (J/g)	$\Delta H_{H,ex}$ (J/g)
H:B = 3.5:96.5	130.0	81.7	86.5	139.2	6.0	3.4
H:B = 7.1:92.9	129.8	80.5	83.2	139.0	11.9	6.8
H:B = 7.7:92.3	129.4	81.5	82.7	138.7	14.2	7.4
H:B = 7.9:92.1	130.1	78.4	82.5	139.1	13.1	7.6
H:B = 8.2:91.8	129.9	78.3	82.2	138.7	12.7	7.9
H:B = 8.4:91.6	129.9	81.7	82.1	139.1	12.4	8.1
H:B = 14.4:85.6	130.0	73.6	76.7	139.1	21.6	13.8
H:B = 18.4:81.6	130.0	70.3	73.1	138.7	24.8	17.7
H:B = 19.6:80.4	130.0	69.6	72.0	139.2	24.4	18.8
H:B = 19.7:80.3	129.4	69.3	71.9	139.0	27.0	18.9
H:B = 20.5:79.5	129.9	69.3	71.2	139.2	26.3	19.7
H:B = 25.4:74.6	130.0	65.4	66.8	139.0	26.6	24.4
H:B = 27.3:72.7	129.9	62.1	65.1	139.4	34.2	26.3
H:B = 38.2:61.8	130.0	52.5	55.4	139.3	38.4	36.7
H:B = 44.7:55.3	130.3	48.9	49.5	140.3	45.8	43.0
H:B = 44.7:55.3	130.5	46.4	49.5	140.3	45.8	43.0
H:B = 44.8:54.6	130.0	46.2	49.4	139.4	49.4	43.1
H:B = 45.4:54.6	130.5	47.2	48.9	140.5	46.8	43.7
H:B = 60.8:39.2	130.4	33.0	35.1	140.5	60.9	58.5
H:B = 62.0:38.0	130.3	31.6	34.0	140.0	62.9	59.6
H:B = 68.6:31.4	130.9	26.8	28.1	140.9	68.4	66.0
H:B = 70.0:30.0	130.4	28.1	26.9	140.4	67.4	67.3
H:B = 80.9:19.1	130.4	15.8	17.1	141.1	80.7	77.8
H:B = 81.3:18.7	130.6	14.6	16.7	140.9	80.0	78.2
H:B = 85.2:14.8	128.5	9.2	13.3	138.3	84.5	82.0
H:B = 88.2:11.8	130.2	9.9	10.6	141.1	85.4	84.8
H:B = 90.4:9.6	128.6	6.1	8.6	138.6	90.1	87.0
H:B = 92.8:7.2	128.7	4.2	6.4	138.4	90.5	89.3
H:B = 94.9:5.1	131.5	2.9	4.6	140.8	91.6	91.3

become superimposed, while, with low  $\beta$ , a small exothermal effect between the melting peaks occurs which does not allow their right integration. With composition (H:B) 60:40, on the contrary, the best heating rate has proved to be 20 K/min, and with composition (H:B) 80:20, 30 K/min.

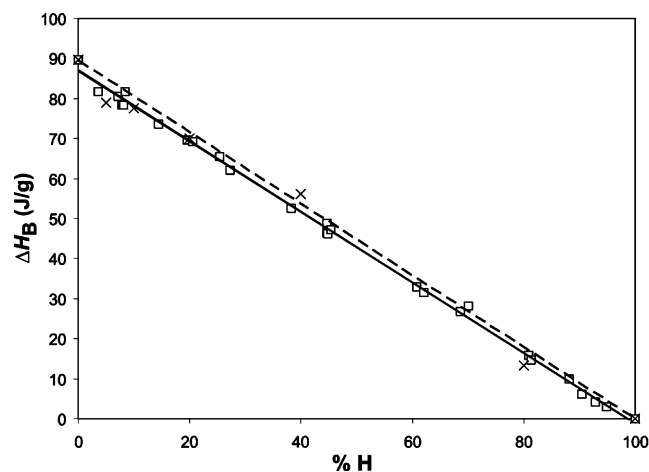
In Table 2 the onset temperatures ( $T_B$ ,  $T_H$ ) and the measured ( $\Delta H_{B,mea}$ ,  $\Delta H_{H,mea}$ ) enthalpy changes of the two melting peaks are reported together with the ones expected on the basis of the mixtures composition ( $\Delta H_{B,ex}$ ,  $\Delta H_{H,ex}$ ). Also in this case, the expected values have been calculated taking into account as melting enthalpies of the two polymorphs B and H  $89.6 \pm 3.6$  and  $96.2 \pm 3.0$  J/g, respectively. From Table 2 it can be inferred that: (a) the experimental values measured for the melting peak of B polymorph nearly always agree with the expected ones, within the experimental error; (b) the experimental melting enthalpies of the H polymorph are significantly higher than the expected values for H content up to 20%. Such a difference is no longer present at higher H content. Since the differences observed between experimental and expected values are definitely higher in the case of ground mixtures with respect to what happens in the case of the physical mixtures, it can be maintained that grinding plays a role in causing the polymorphic transition. Thus, this method of preparation of binary systems must be rejected.

### 3.3. Binary systems directly prepared in the DSC pans

The calorimetric traces of the binary systems directly prepared in DSC pan are qualitatively similar to those already described for the physical mixtures. Also in this case the measurements have been performed at different  $\beta$ , depending on the composition, in order to obtain the separation most suitable for the peaks integration.

In Table 3 the onset temperatures ( $T_B$ ,  $T_H$ ) and the measured ( $\Delta H_{B,mea}$ ,  $\Delta H_{H,mea}$ ) enthalpy changes of the two melting peaks are reported together with the ones expected on the basis of the systems composition ( $\Delta H_{B,ex}$ ,  $\Delta H_{H,ex}$ ). It is evident that the differences

between the measured and expected enthalpies for the melting of B polymorph are very low, although the measured values are always but in one case lower than the expected ones. The differences between measured and expected enthalpies for the melting of H polymorph are, both in absolute and relative terms, mostly higher than those relating to B polymorph. It is worth underlying that the measured melting enthalpy of H form is always higher than the expected one. Fig. 3 shows the melting enthalpies of B form as a function of the H form content both for the physical mixture (prepared in turbula) and for the binary systems prepared in DSC pan. The linear regression of the two series of data are practically superimposed. Since the points obtained with the physical mixtures and with the systems directly prepared in DSC pan are undistinguishable, it is possible to interpolate them with a single straight line



**Fig. 3.** Melting enthalpy of form B measured for binary systems prepared in DSC pan (squares) and for physical mixtures prepared in turbula (crosses) as a function of the mass percentage of H form, their linear regression (solid line) and behaviour expected on the basis of the composition of the system (dotted line).

(solid line in Fig. 3). The equation of this straight line is:

$$\Delta H_B = -0.882\%H + 86.7 \quad (R^2 = 0.9968) \quad (1)$$

The theoretical straight-line (dotted line in Fig. 3) is:

$$\Delta H_B = -0.896\%H + 89.6 \quad (R^2 = 1)$$

The agreement between the behaviour of the two series of samples (physical mixtures and binary systems prepared in the DSC pan) indicates that neither the preliminary treatment of the pure samples neither the mixing in turbula is responsible for the polymorphic transition. We believe, on the contrary, that a limited extent of H phase formation is due to heating during DSC run. Indeed it has been observed that the major deviation from the expected enthalpy occurs with measurements performed at low heating rate. Such measurements had to be performed on samples with low H content in order to succeed in the separation of the polymorphs melting peaks. As B form undergoes melting (128.9 °C) the solid particles of H form grow from the melt and the H content of the system increases and this in turn causes the greater melting enthalpy of the H form with respect to the expected value. Owing to this behaviour, the H form content cannot be determined from its melting peak but from that of B modification. In particular, the quantification of the polymorph H in mixture with the B form can be performed through the calibration straight-line (Eq. (1)) obtained from the melting enthalpy values measured for the polymorph B and its nominal content in systems of known composition. The method is able to accurately determine the presence of 3% impurity of H in a binary system.

#### 4. Conclusions

Experimental evidences have determined that the grinding of samples containing both solid forms of nateglinide induces the transition from polymorph B to H. The enrichment of the binary systems with H form is caused also by heating. The quantification of H polymorph in mixture with B can be performed by means of DSC through the calibration straight-line obtained from the melting enthalpy values of B polymorph and the H nominal content in mixtures of known composition.

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