



New solid modifications of nateglinide

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ABSTRACT

New modifications of the antidiabetic drug nateglinide were found and characterized by means of thermal analysis, vibrational spectroscopy and X-ray powder diffractometry. In particular it has been verified that the product obtained during the final steps of the nateglinide synthesis is the hemihydrate form which melts at about 86 °C provided that the adopted experimental conditions hinder the removal of the crystallization water. Otherwise, if the crystallization water is removed, the hemihydrate transforms to a new anhydrous polymorph that melts at 102.8 °C. The anhydrous polymorph, if stored at room temperature and humidity, gradually changes to H polymorph while, if stored in water vapour saturated atmosphere, it gets back water and reverts to the hemihydrate form. On the contrary, both an isothermal treatment at 80 °C and melt cooling bring to the B polymorph.

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1. Introduction

Since many properties of an active principle, such as solubility, stability and technological characteristics, may be related to the solid form of the pharmaceutical substance, the investigation of the polymorphic behaviour is an essential step of the preformulation study [1–3]. Often the thermodynamically stable modification is chosen for the drug development but this criterion has not to be taken as the general rule. Nateglinide, (-)-N-[(trans-4-isopropylcyclohexane carbonyl)-D-phenylalanine], is a drug used to lower sugar levels in patients with type 2 diabetes by stimulating release of insulin [4] that can exist in three polymorphs [5]. In our previous work [6] we defined the relationships of relative stability among the three known nateglinide polymorphs: in particular it has been found that they are in relationship of monotropy i.e. the S form, the highest-melting form, is the only stable, while the polymorphs B and H are metastable forms. Furthermore it has been assessed that the S polymorph can crystallize from the melt or also by an isothermal treatment of B or H forms at temperatures lower than their melting points. While H form is now claimed by active patent [7], the B and S forms are commercially free even if thorough clinical studies on the S polymorph have not yet been completed. So, for the pharmaceutical industry it would be important to obtain the pure B polymorph. A good synthetic route has been successfully

developed on a laboratory scale, but, moving to production scale, a transition from B to H form occurs during the final steps of drying and milling. Thus, a deeper knowledge of the behaviour of the wet product obtained during the final synthesis steps seems to be a logical starting point of the investigation whose results are presented in the following.

2. Materials and methods

The analyses have been performed on the sample obtained dissolving nateglinide in a biphasic system of diluted KOH water solution, methanol and toluene and precipitating it by adding diluted HCl water solution at 15 °C (AMSA S.p.A., Como, Italy). The isolated wet product has been suspended in water to remove KCl. We will refer to it in the following as W sample.

Thermal analyses were carried out with a TGA Q5000 IR apparatus and a DSC Q2000 apparatus both interfaced with a TA 5000 data station (TA Instruments, USA). Thermogravimetric curves were recorded in a standard platinum holder, under a nitrogen flow (3 L h⁻¹) at a heating rate (β) ranging from 3 K min⁻¹ to 50 K min⁻¹. The DSC instrument was calibrated using ultrapure (99.999%) indium (m.p. = 156.6 °C; $\Delta H = 28.54 \text{ J g}^{-1}$) as standard. The calorimetric measurements were conducted both in open and in closed standard aluminum pans under a nitrogen flow (3 L h⁻¹) at different heating rates (3–50 K min⁻¹). All numeric figures from thermal measurements are an average of three or more experiments.

FT-IR spectra by diffuse reflectance were obtained with a Nicolet FT-IR 730 Spectrometer equipped with a DRIFT collector by Spectra Tech, UK. Samples were thoroughly mixed with anhydrous KBr

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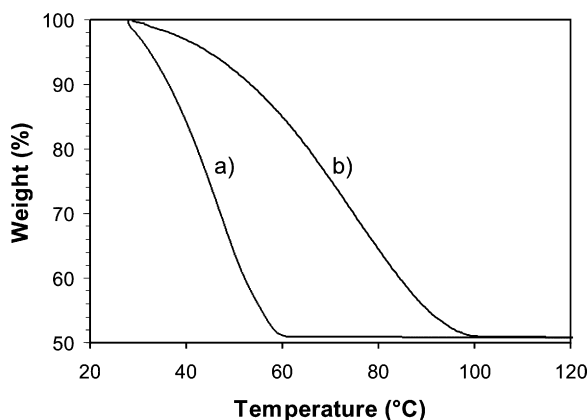


Fig. 1. TG curves of W sample under nitrogen flow at different heating rates: 3 K min⁻¹ (curve a) and 30 K min⁻¹ (curve b).

and the spectra were collected by co-adding 1024 scans in the 4000–400 cm⁻¹ range at 4 cm⁻¹ resolution.

X-ray diffraction patterns (CuK_α radiation; voltage of 40 kV and current of 30 mA) were collected with a powder diffractometer Bruker D5005 (Siemens, Germany) equipped with a θ - θ vertical goniometer and a Position Sensitive Detector (PSD, Braun). The spectra have been recorded in the step scan mode (step: 0.015°, counting time: 0.5 s) in the angular range $2\theta = 4$ –30° at room temperature.

3. Results and discussion

3.1. TG measurements

The W sample, heated in thermobalance under nitrogen flow, loses mass from the very beginning of the measurement. The total mass change is $-49.2 \pm 1.2\%$ and it takes place in a single step. We assume it is due to water removal. When the heating rate is 3 K min⁻¹, the step ends at about 60°C (Fig. 1a), while at 30 K min⁻¹, as expected, the mass change is completed at higher temperatures (about 100°C, Fig. 1b).

The mass loss process of W has been studied with a different method. Namely a W sample has been put on an analytical balance at 23°C and 28% relative humidity. The sample starts losing mass and, within two hours, it loses about 52% of the starting mass. By keeping the sample exposed to laboratory atmosphere for times up to 68 h, no further mass loss has been recorded even if, by submitting the sample to a TG analysis, it has been found that it still contains $2.9 \pm 0.2\%$ of water (Fig. 2) that can be removed simply by means of an isothermal stage of 5 min at 30°C under nitrogen flow. The difference between the total water content measured with a thermobalance ($\approx 49\%$) and that measured with the analytical balance ($\approx 55\%$) can be accounted for by considering that the W sample is particularly sensible to atmosphere water vapour content. Indeed, it loses water as a consequence of its exposure to laboratory atmosphere. Even more so, it loses water still faster, before the start of the measurement, in the TG furnace where it is exposed to a dry nitrogen flow at about 29°C (the lowest working furnace temperature). It is unavoidable that some time elapses between the moment in which the sample is taken from the container and the moment in which the data acquisition starts: such a delay does not exist when the sample is put on an analytical balance.

A W sample equilibrated at ambient temperature and water partial pressure contains 2.9% of water and in the following will be referred to as PD (PD \equiv Partially Dehydrated). A sample with no residual water in the following will be referred to as CD (CD \equiv Completely Dehydrated); this same sample, when equi-

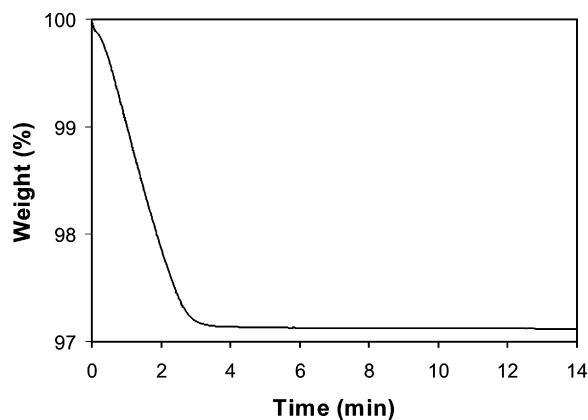


Fig. 2. TG curve of W sample exposed to laboratory atmosphere for 68 h at 23°C and 28% relative humidity (isothermal at 30°C under nitrogen flow).

brated in wet atmosphere, acquires again 2.6% of water and it will be referred to as R (R \equiv Rehydrated).

We note that the values 2.6–2.9% agree, within the experimental error, with that expected for the hemihydrate nateglinide (2.7%). Thus, TG measurements strongly suggest that the W, the PD and the R samples contain 2.6–2.9% of crystallization water and are the same solid phase: hemihydrate nateglinide. The only difference between W sample and sample PD and R is the amount ($\approx 50\%$ by mass) of surface water present in sample W.

3.2. DSC measurements

DSC traces are definitely different with changing heating rate and pan configuration. In particular the results of DSC measurements will be reported separately for the runs performed in open or in closed pan i.e. under conditions that allow or hamper (controllare) the water release process.

3.2.1. DSC measurements in open pan

3.2.1.1. Heating rate: 3 K min⁻¹. The DSC trace of the W sample in open pan at 3 K min⁻¹ in nitrogen flow is shown in Fig. 3. It is different from the one of the pure B polymorph [6] for the presence of an initial endothermic drift due to water removal at low temperatures (it is not an actual peak because the sample, as previously noted, soon loses water and the record of an initial baseline is not possible) and to an endo-exothermic peak at $101.4 \pm 0.2^\circ\text{C}$. This last effect is followed by the peaks that appear in the DSC trace of the B form: endothermic peak of melting ($127.5 \pm 0.7^\circ\text{C}$), exothermic peak due to the crystallization of S form from liquid phase

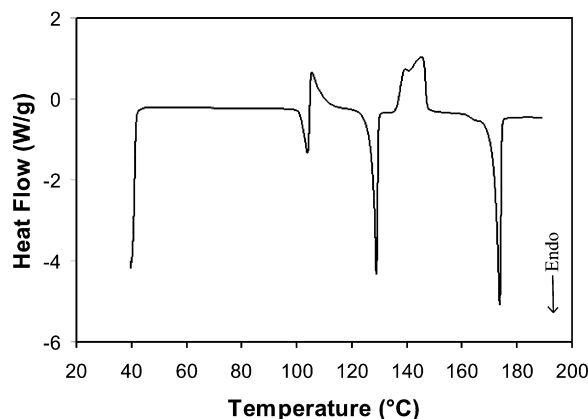


Fig. 3. DSC curve of W sample (open pan, nitrogen flow, 3 K min⁻¹).

Table 1
Quantitative data from thermal measurements performed at 3 K min^{-1} in open pan obtained for W, PD, CD and R samples and corrected on the basis of system water content.

Sample	$T_{e,B}$ ($^{\circ}\text{C}$)	ΔH_B (J g^{-1})	$T_{e,exo}$ ($^{\circ}\text{C}$)	ΔH_{exo} (J g^{-1})	$T_{e,S}$ ($^{\circ}\text{C}$)	ΔH_S (J g^{-1})
W	127.5 ± 0.7	73.8 ± 4.9	136.4 ± 0.9	-97.2 ± 3.1	171.6 ± 0.4	97.5 ± 0.7
PD	127.2 ± 0.8	72.8 ± 5.4	136.4 ± 1.7	-96.8 ± 2.1	171.8 ± 0.3	94.9 ± 1.8
R	127.3 ± 0.7	65.4 ± 3.1	135.6 ± 1.2	-91.6 ± 3.2	172.2 ± 0.4	90.3 ± 1.3
CD	127.1 ± 0.7	68.4 ± 4.4	136.1 ± 1.4	-93.3 ± 2.5	172.1 ± 0.5	92.6 ± 1.5
B	129.2 ± 0.6	85.5 ± 2.4	140.5 ± 0.1	-100.4 ± 1.4	172.2 ± 0.9	101.2 ± 1.5

The thermal data measured for B sample [5] are reported for comparison.

($136.4 \pm 0.9^{\circ}\text{C}$) and melting peak of S form ($171.6 \pm 0.4^{\circ}\text{C}$). The PD and R samples give a DSC trace like that now described, when β is 3 K min^{-1} . For all these samples (Table 1) the enthalpy changes of the B form melting, of crystallization and melting of S form (corrected considering the sample water content) are always lower than the values measured for B sample [6]. In particular, the difference is higher for the B form melting.

The DSC trace of the CD samples is different from that of the PD ones: the initial endothermic drift due to the dehydration does not show up and a weak endothermic peak appear at $133.3 \pm 1.2^{\circ}\text{C}$ i.e. very close to the melting temperature of H form (Fig. 4). As this peak is soon followed by the exothermic effect of crystallization its exact integration is not possible. However, the amount of heat involved corresponds to a few units of J/g and increases with sample ageing (stored in closed container).

3.2.1.2. Heating rate: 30 K min^{-1} . The DSC trace of the W sample at 30 K min^{-1} (open pan, nitrogen flow) shows (Fig. 5), after the endothermic effect due to water removal, an endothermic peak at $102.8 \pm 0.18^{\circ}\text{C}$ sometimes followed by a very weak endothermic

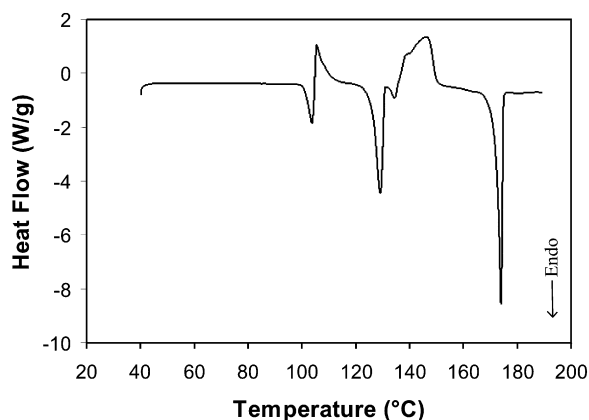


Fig. 4. DSC curve of CD sample (open pan, nitrogen flow, 3 K min^{-1}).

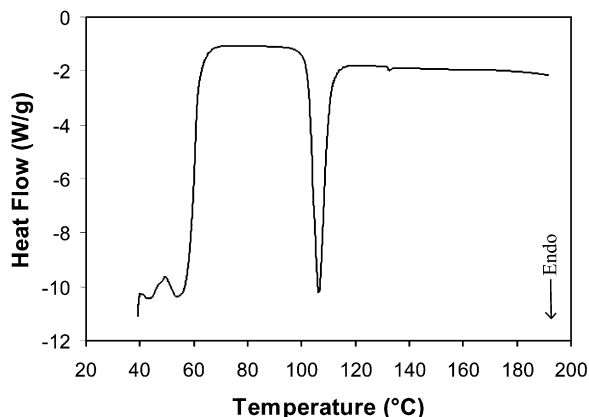


Fig. 5. DSC curve of W sample (open pan, nitrogen flow, 30 K min^{-1}).

Table 2
Enthalpy change (ΔH_{133}) measured for the DSC peak at 133°C with CD samples.

Storing time of CD sample	ΔH_{133} (J g^{-1})
1 day	0.9
15 days	3.4
21 days	5.4
40 days	8.7

mic effect (at most 0.9 J/g) at 127°C (temperature corresponding to melting of B form). The DSC traces of PD and R samples are very similar to that obtained with the W sample. The enthalpy change of the peak at 102.8°C , correct on the basis of sample water content, is $58.5 \pm 2.3\text{ J g}^{-1}$.

The DSC trace of the CD samples distinguishes from that of the PD samples for the presence of a weak peak at $133.2 \pm 0.1^{\circ}\text{C}$, temperature corresponding to that of H form melting (Fig. 6). As already observed for the DSC measurements performed at 3 K min^{-1} , also when β is 30 K min^{-1} , the entity of this peak increases with sample ageing (stored in closed container) as indicated in Table 2.

3.2.1.3. Discussion of DSC measurements in open pan at different heating rates. The described experimental picture suggests that all the samples are a new crystalline form, different from the B form. As it has been seen in the TG section, W sample distinguishes from the PD and the R samples only for “not bound” water content. The same TG measurements suggest that it is a hemihydrate form (in the following referred to as form E). As a consequence of crystallization water removal, a new anhydrous polymorph is obtained which will be named A form and which melts at 102.8°C . The sample shows a different thermal behaviour with changing heating rate:

- With low heating rate (3 K min^{-1}), the A form melts and soon crystallizes to B form (exothermic peak) which then melts. Indeed, the DSC trace after 120°C overlaps with that of a pure B sample (Fig. 7, curve a).

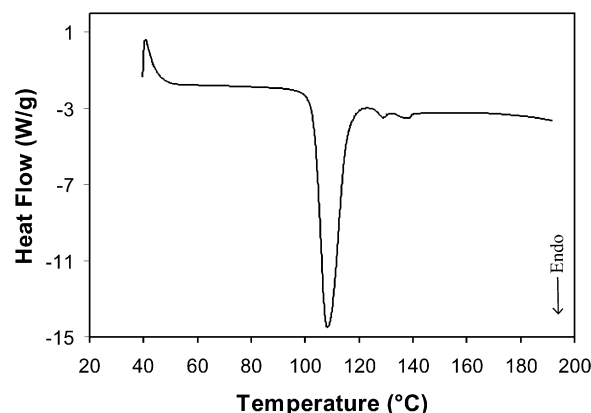


Fig. 6. DSC curve of CD sample (open pan, nitrogen flow, 30 K min^{-1}).

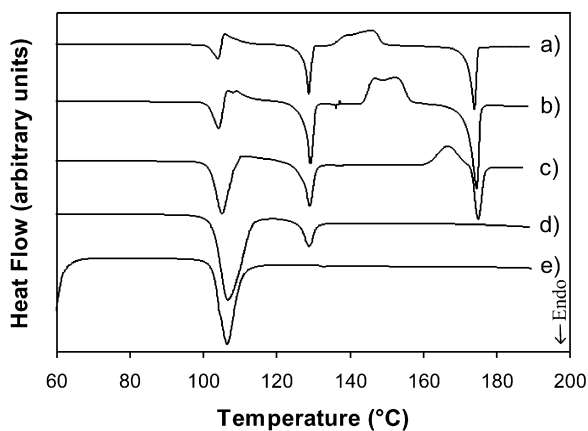


Fig. 7. DSC curves of PD sample at different heating rates (open pan, nitrogen flow): 3 K min⁻¹ (curve a), 5 K min⁻¹ (curve b), 10 K min⁻¹ (curve c), 20 K min⁻¹ (curve d) and 30 K min⁻¹ (curve e).

- When the sample is fast heated (30 K min⁻¹), after melting of the A form, no other peaks are present: the crystallization to B polymorph does not happen due to kinetic reasons (Fig. 7, curve e).

Fig. 7 shows the DSC curves obtained with open pans and at different heating rates (3–30 K min⁻¹) on PD samples. It is evident that the area of the melting peak of A form increases with increasing heating rate while that of the melting peak of B form decreases. This behaviour is exactly predictable on the basis of the proposed model.

It has been put into evidence that the melting enthalpy of B form and the enthalpies of crystallization and melting of S form are slightly lower than expected. In our opinion such behaviour is due to a small amount of amorphous nateglinide in the analyzed sample.

3.2.1.4. Cyclic measurements. With the aim to evaluate the reversibility character of the melting peak of E at 102.8 °C a cyclic measurement has been performed as follows: - first heating at 30 K min⁻¹ up to 120 °C (temperature corresponding to the closing of A melting peak); - cooling down to room temperature; - second heating at 3 or 30 K min⁻¹. During cooling an exothermic peak is recorded for the crystallization of B polymorph which melts in the following scan. Therefore, the melting peak of A form is not reversible.

3.2.1.5. Isothermal measurements. In Fig. 8 the effect of an isothermal step of 1, 3, and 12 h at 80 °C (temperature that precedes the melting of A form) on the following ramp recorded at 30 K min⁻¹ is shown: with increasing the annealing time, the A form melting peak area decreases up to disappear, while the one of B polymorph melting increases. After 12 h isothermal step the enthalpy change of the last one reaches the value expected for the pure B polymorph. Thus, the annealing at 80 °C allows the complete transformation from A polymorph to B polymorph. The quantitative analysis suggests that the annealing at 80 °C allows also the crystallization of that small amount of amorphous nateglinide present in the W samples.

3.2.2. DSC measurements in closed pan

3.2.2.1. Heating rate: 3 K min⁻¹. The DSC measurements performed in closed pan on the W sample are difficult to interpret for the big amount of water that evaporates from the sample causing irregular thermal effects. DSC curves nearly identical to those obtained, by the same heating rate, with open pans (Fig. 9) are obtained with the PD and the R samples. An endothermic peak with onset temperature of 45.0 °C due to crystallization water removal from the sample

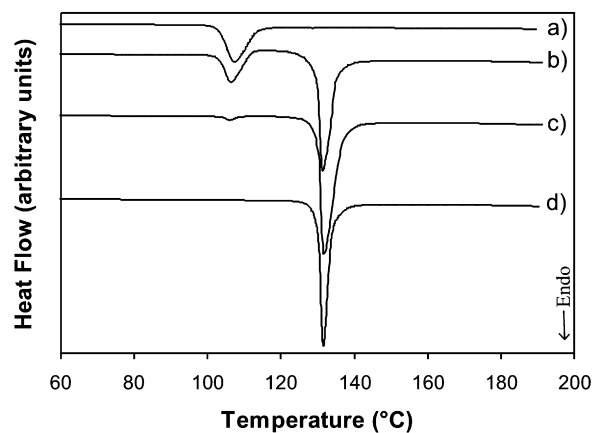


Fig. 8. DSC curves of PD sample as a function of the annealing time at 80 °C (open pan, nitrogen flow, 30 K min⁻¹): 0 h (curve a), 1 h (curve b), 3 h (curve c) and 12 h (curve d).

is recorded. The peak area is 71.5 ± 1.9 J for gram of nateglinide corresponding to 44 kJ for mol of water. This value well agrees with the value expected for the breaking of hydrogen bonds with water. At higher temperatures the same effects recorded in open pan with the same β are present.

3.2.2.2. Heating rate: 30 K min⁻¹. When the PD and the R samples are heated in closed pan at 30 K min⁻¹, a broad endothermic effect at 84.8 ± 0.5 °C is recorded. It is followed by the typical B polymorph melting peak at 127 °C (Fig. 10). For the first effect, an enthalpy

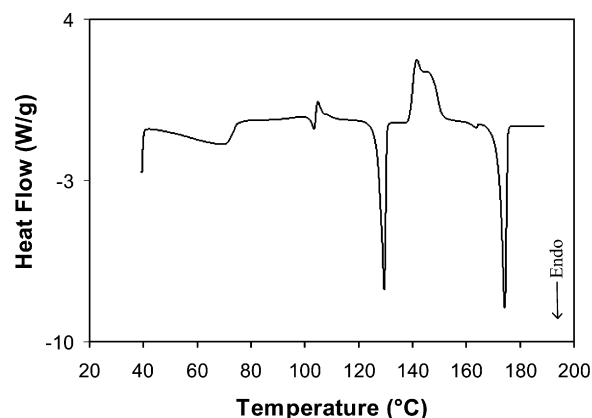


Fig. 9. DSC curve of PD sample (closed pan, nitrogen flow, 3 K min⁻¹).

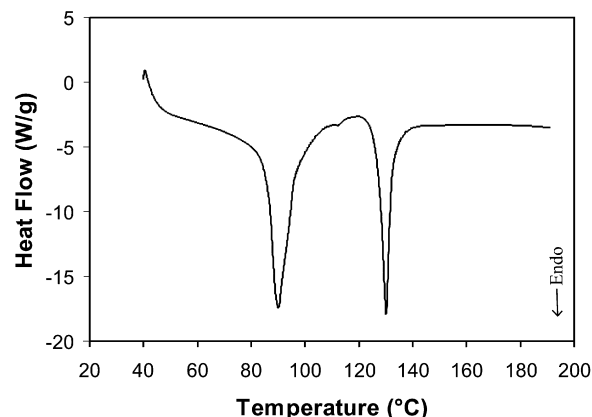


Fig. 10. DSC curve of PD sample (closed pan, nitrogen flow, 30 K min⁻¹).

change of $105.9 \pm 0.2 \text{ J g}^{-1}$ is measured. We consider unlikely the attribution of this peak only to water removal from the sample. In fact, the dehydration enthalpy obtained by relating the value measured by the instrument to the water content (about 66 kJ/mol of water) is surely too high compared to the value we can expect on the basis of the hydrogen bonds breaking. The integration of the peak at 127°C indicates that an amount of B form equal to about half of the mass of the analyzed sample melts.

3.2.2.3. Discussion of DSC measurements in closed pan at different heating rates. The behaviour of the samples in closed pan may be explained considering that, with such a configuration, the sample dehydration is strongly hampered and shifted to higher temperatures. In this respect, it is important stress that the pan is closed, though not hermetically sealed, by means of a pressure application on the lid that press the powder reducing the pan dead space. Thus, the water vapour pressure builds up in the pan becoming soon high and, as a consequence, most of the hemihydrate form at 30 K min^{-1} melts before loosing the crystallization water. At the same time of the melting, the water evaporation takes place. According to this model, the endothermic peak at 85°C is caused by partial dehydration of the sample, by melting of the hemihydrate form and by water evaporation from the melt.

Such an interpretation has been supported by performing TG measurements on samples put in a closed pan identical to that used in the DSC measurements. At low β (3 K min^{-1}) a mass change step (2.9%) is present in the same temperature range of the DSC peak due to water release and this fact confirms that the sample pan is not hermetic: the water release from the crystal lattice causes an high vapour pressure which makes the pan leaks. At high β the mass change step is shifted towards higher temperatures: in particular, at 30 K min^{-1} , the mass change step occurs in a temperature range which matches that of the DSC peak with onset temperature of 85°C , while, at 50 K min^{-1} , the mass change is slightly delayed with respect to the DSC peak. Therefore, at high heating rate, almost all the nateglinide melts as hemihydrated form and, at the same time, water evaporates from the melts.

The hypothesis according to which the peak at 85°C originates from a melting process too is supported by a cyclic DSC measurement with a first heating at 30 K min^{-1} up to 115°C , a cooling down to room temperature and a second scan at 30 K min^{-1} . During cooling an exothermal peak is recorded due to crystallization of B form which melts in second scan with an enthalpy change corresponding to the one expected for the pure B polymorph.

3.3. XRPD measurements

The W sample is made of wet and sticking granules which make the sample preparation for X-ray analysis very difficult. Furthermore, the pattern of the W sample is difficult to interpret for the presence of weak and very broad peaks. Thus, we will not consider the patterns of these samples in the following.

The peaks are, on the contrary, intense and well separated in the PD samples. The patterns of all these samples, in any condition they have been obtained, are similar. The diffraction effects are present in angular positions different from those of the pure B and H polymorphs (Fig. 11). This behaviour confirms our hypothesis: the PD samples are a new crystalline modification, the hemihydrate form.

The CD sample shows a new pattern: its peaks may be attributed to none of the known phases. Also these results agree with the model inferred from the thermal measurements: the hemihydrated form, as a consequence of the removal of crystallization water, changes to a new polymorph (Fig. 11).

The R sample shows the same diffraction effects as the PD sample. For this sample the TG measurements indicated the water

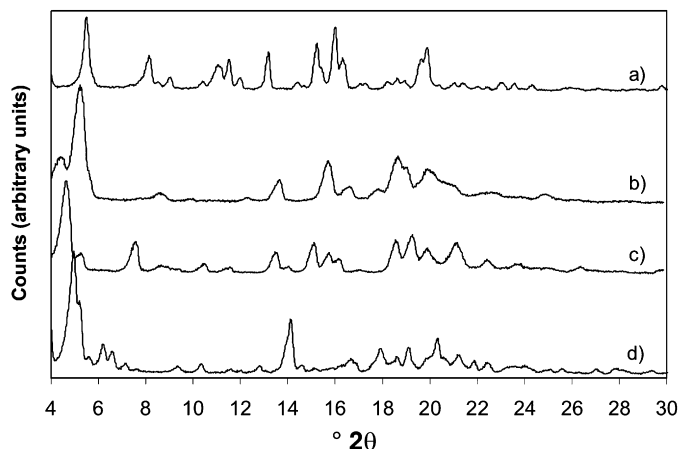


Fig. 11. XRPD patterns of H polymorph (curve a), CD sample (curve b), PD sample (curve c) and B polymorph (curve d).

content of 2.6%. This experimental evidence shows that the anhydrous A form in presence of humidity changes again to the hemihydrated form.

The pattern of the W sample (and that of the PD samples) after 12 h annealing at 80°C becomes the same as that of the pure B polymorph (Fig. 12). Also this experimental evidence confirms the DSC results: the annealing at 80°C leads to B form.

3.4. FT-IR measurements

The FT-IR measurements show that the spectroscopic properties of the W are the same as that of the PD and the CD samples but they are different from those of the pure B polymorph. In Fig. 13 the spectra of the W sample are compared to that of the B polymorph. The main differences are the following:

- (i) the NH stretching vibration is at 3357 cm^{-1} in the B polymorph sample spectrum while it is at 3310 cm^{-1} in the W sample spectrum;
- (ii) the carbonyl group of the carboxylic function stretching vibration is at 1741 cm^{-1} (spectrum a) while it is split in the W sample spectrum (1762 and 1731 cm^{-1});

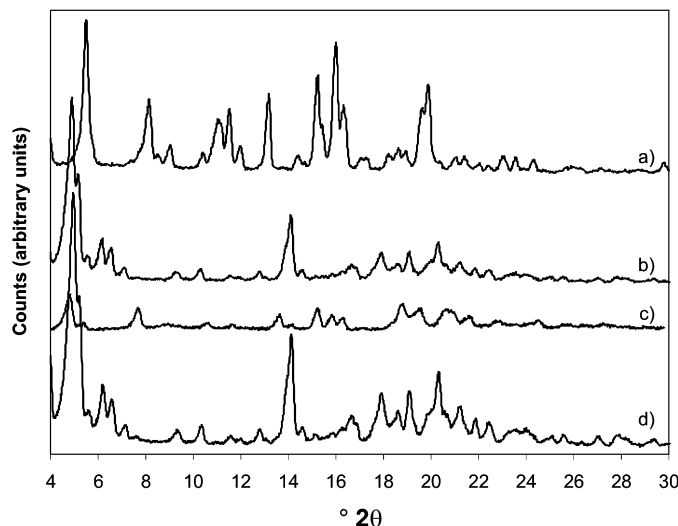


Fig. 12. XRPD patterns of H polymorph (curve a), W sample annealed 12 h at 80°C (curve b), W sample (curve c) and B polymorph (curve d).

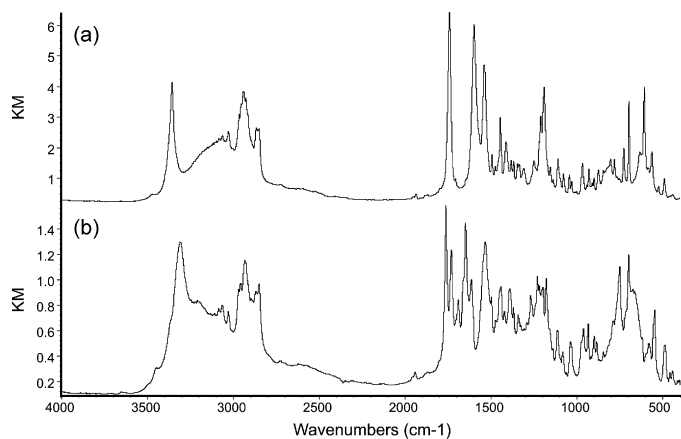


Fig. 13. FT-IR spectra of B polymorph (a) and W sample (b).

- (iii) the benzene ring stretching vibration at 1598 cm^{-1} (spectrum a) is absent in the spectrum b;
- (iv) the C–N–H bending vibration (amide band II) is at 1538 cm^{-1} (spectrum a) and at 1532 cm^{-1} (spectrum b);
- (v) differences in the region below 900 cm^{-1} .

Since for the spectroscopic analysis it is necessary to prepare a mixture with KBr which is highly hygroscopic and to expose it to a nitrogen flow for several minutes before starting the measurement, it is easy to imagine that in this time the complete sample dehydration takes place. Thus, the sample W transforms, during the analysis, into the anhydrous form A. Hence the spectral differences reported under points (i)–(v) are to be intended as spectral differences between spectra of A and B form since the FT-IR technique does not allow, to record the spectrum of the hemihydrated form (E).

The FT-IR spectrum of the W sample annealed 12 h at 80°C is the same as that of B sample. These results confirm the hypothesis that the annealing at 80°C leads to the polymorphic change from A to B form.

4. Conclusions

The present study has allowed to gain a deep knowledge of the behaviour of the wet product obtained during the final synthesis steps of nateglinide so that it is possible to reasonably select the experimental conditions which address the synthesis route to the formation of a specific solid form. As a matter of fact, the correlated analysis of TG, DSC, XRPD and FT-IR measurements leads to maintain that the W sample as such and the partially dehydrated one are the hemihydrate form of nateglinide (E) which melts at about 86°C and contains a low amount of amorphous sample. As a consequence of the crystallization water release, the hemihydrate form changes to a new polymorph (A) with melting point of 102.8°C . Either melt cooling and an isothermal treatment at 80°C of the A form, cause the crystallization of the B polymorph takes place. The new anhydrous polymorph, if stored at room temperature and humidity, gradually changes to H polymorph while, if stored in water vapour saturated atmosphere, it gets back water and changes into hemihydrate form (E).

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