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Evidence for polymorphism in glisentide

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Abstract

The polymorphism of glisentide has been investigated. Three polymorphs (I, II, III) have been prepared by recrystallization from different solvents and other polymorphic form (IV) was obtained by heating polymorph III at 100°C. In addition, two 1:1 stoichiometric solvates containing carbon tetrachloride and dioxane have been crystallized and finally, an amorphous solid has been obtained. It has been observed that the polarity of the recrystallisation solvent and its ability to form hydrogen bonds have a great influence on the polymorphism of glisentide. The different solid forms of glisentide have been characterized using X-ray diffraction analysis, differential scanning calorimetry (DSC), thermogravimetric analysis (TGA), IR spectroscopy and optical microscopy. The recrystallization of polymorph I in melted form II and also the transition of form III–IV have been detected by DSC and X-ray diffraction analysis.

Keywords: Glisentide; Polymorphism; Solvate

1. Introduction

The investigation of drug polymorphism is an important step in any preformulation study because polymorphism may have a considerable influence on solid state physical properties which can modify the biopharmaceutical and technological behaviour of a drug.

Glisentide, ($C_{22}H_{27}O_5N_3S$), *N*-[2-[4-[[[(cyclopentylamino)carbonyl]amino]sulfonyl]phenyl]ethyl]-2-methoxy-benzamide, is an oral antidiabetic agent that belongs to the sulfonylurea group, its chemical structure (Fig. 1) and its inclusion behaviour is closely related to glibenclamide (Zornoza et al., 1998). Polymorphism is widespread among sulfonylureas as these compounds present chemical groups that are able to form hydrogen bonds and dipolar interactions which are often involved in

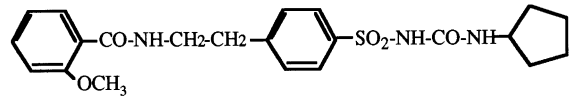

Fig. 1. Chemical structure of glisentide.

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the formation of different polymorphic structures. Some of these sulfonylurea compounds which have been found to exhibit polymorphism are acetohexamide (Girgis-Takla and Dakas, 1989), tolbutamide (Al-Saieq and Riley, 1981), chlorpropamide (Al-Saieq and Riley, 1982) and glibenclamide (Suleiman and Najib, 1989).

The aim of this study was to investigate the existence of different polymorphic forms of glisentide. Polymorphs and solvates have been obtained by precipitation from different solvents under variable conditions and they have been characterized by X-ray diffractometry, IR spectroscopy, differential scanning calorimetry (DSC), thermogravimetric analysis (TGA) and optical microscopy.

2. Materials and methods

2.1. Materials

Glisentide was kindly supplied by Uriach-Biohorm (Barcelona, Spain).

The crystallizations have been carried out in ethanol, acetone, methanol, acetonitrile, ethyl acetate, *N*,*N*-dimethylformamide, dioxane and carbon tetrachloride, all these solvents were of analytical reagent grade.

2.2. Procedures for obtaining polymorphs and solvates of glisentide

2.2.1. Form I

A saturated solution of glisentide in ethanol at 70°C was cooled at room temperature. This form was also obtained using methanol, acetone, acetonitrile and *N*,*N*-dimethylformamide as recrystallisation solvents. The crystals precipitated in three days and were isolated by filtration. This is the form provided by the manufacturer.

2.2.2. Form II

A saturated solution of glisentide in ethyl acetate at 72°C was allowed to cool at room temperature and the crystals precipitated within 5 days; however, this method produces small amounts of polymorph II due to the low solubility of the drug in this solvent. An alternative method to obtain this form consisted of dissolving 100 mg of glisentide in 4.5 ml of acetone at 40°C which were added to 20 ml of ethyl acetate at 72°C, the solution was maintained at this temperature during 10 min, then, it was slowly cooled using a temperature program of 10°C/h approximately.

2.2.3. Form III

It was prepared by rapid cooling of a saturated solution of glisentide in dioxane at 80°C in a refrigerator at 4°C and the crystals precipitated in 6 h.

2.2.4. Form IV

This polymorph was obtained by heating form III, amorphous glisentide and the solvate with carbon tetrachloride at 100°C.

2.2.5. Solvate with dioxane

A saturated solution of glisentide at 80°C was slowly cooled using a temperature program of 10°C/h approximately. The crystals precipitated in 5 h.

2.2.6. Solvate with carbon tetrachloride

A saturated solution of glisentide in carbon tetrachloride at 70°C was slowly cooled using a temperature program of 10°C/h approximately and the crystals precipitated within 3 days. This method produces small amounts of solvate due to the low solubility of the drug in this solvent, so an alternative method similar to that proposed for obtaining polymorph II has been used: 100 mg of glisentide were dissolved in 4.5 ml of acetone at 40°C which were added to 20 ml of carbon tetrachloride at 70°C, the solution was maintained at this temperature during 10 min, then it was slowly cooled. The crystals precipitated in 24 h.

2.2.7. Amorphous glisentide

A total of 100 mg of glisentide were dissolved in 4.5 ml of acetone at 40°C and they were added to 20 ml of carbon tetrachloride at 70°C, the solution was maintained at this temperature during 15 min, then it was rapidly cooled at 4°C.

2.3. Characterization of solid phases

X-ray powder diffraction patterns were obtained on a Siemens Kristalloflex 810 diffractometer (Cu–K_α radiation $\lambda = 1.541$ Å; voltage 40 kV; current 20 mA; time constant 4 s; scanning speed 1 deg/cm). The X-ray diffraction patterns of each form were repeated at least four times and a complete reproducibility of the position of the signals (2θ) was obtained but there were problems with the reproducibility of the relative intensities so they have been classified according to the following key: h, high ($I > 0.7I_{max}$); m, medium ($0.70I_{max} > I > 0.35I_{max}$); w, weak ($I < 0.35I_{max}$).

IR spectra were acquired with a Perkin Elmer 681 spectrometer in KBr pellets.

Thermal analysis was carried out in a Setaram DSC-92 system with a scanning rate of 5°C/min.

A Perkin Elmer TGA-7 thermogravimetric system was used to determine the mass loss of solvates at a heating rate of 5°C/min under a nitrogen purge.

The habit of the solid phases was determined using an Olympus BH2S microscope.

3. Results and discussion

3.1. X-ray diffraction analysis

The X-ray diffraction patterns of the different solid phases of glisentide are shown in Fig. 2. Substantial differences in the position and intensity of the peaks (Table 1) indicate the formation of an amorphous solid and six crystalline forms whose subsequent analysis by DSC, IR and TGA suggested the existence of four polymorphs and two solvates.

The X-ray diffraction patterns allow to identify all the crystalline forms of glisentide. Forms I and II appear to have a higher degree of crystallinity than forms III and IV as their diffraction patterns present numerous and sharp reflections. The amorphous phase of glisentide is characterized by the absence of reflections.

The polarity of the recrystallisation solvent seems to have a marked influence on the polymorphism of glisentide because in polar solvents such as ethanol, acetone, methanol, acetonitrile and *N*,*N*-dimethylformamide only form I is crystallized, whilst in non polar solvents such as dioxane, carbon tetrachloride and ethyl acetate a great number of different solid phases is obtained (polymorphs II, III, an amorphous phase, and two solvates). In addition, it seems that the crystallization rate has great influence on obtaining solid phases by recrystallisation from non polar solvents, but it does not affect when polar solvents are used, as form I is obtained anyway using both rapid and slow precipitation rates.

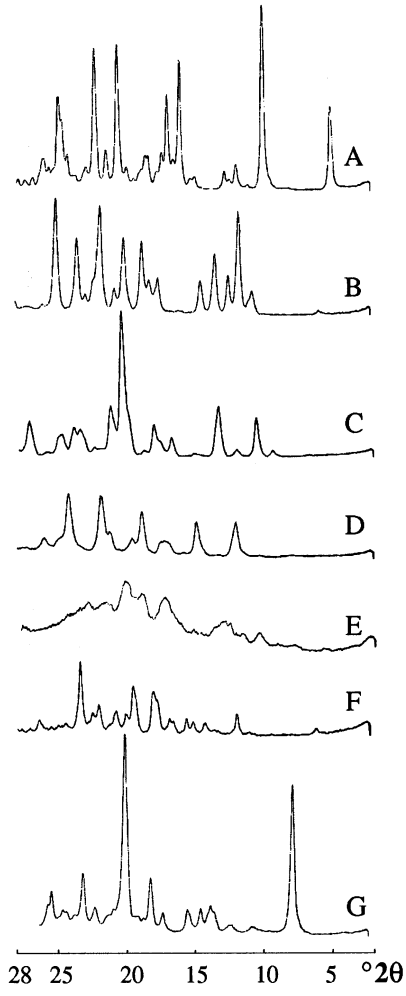

Fig. 2. X-ray diffraction patterns of polymorphs I (A), II (B), III (C), IV (D), amorphous glisentide (E) and the solvates with carbon tetrachloride (F) and dioxane (G).

3.2. Differential scanning calorimetry

The differences observed in the DSC curves of the different solid phases (Fig. 3) contribute to characterize them and it is also possible to detect phase transitions induced by heating.

The DSC diagram of form I presents its melting at 171°C. Polymorph II exhibit a first endothermic at 160°C which corresponds to its melting, it is followed by a small exothermic peak due to the recrystallization of form I in the melted product and finally, the melting of polymorph I gives rise to a peak at 172°C. In order to confirm the crystallization of phase I, polymorph II has been heated at 160°C and the diffraction pattern of this heated product was found to present the characteristic reflections of form I. The melting temperatures of forms I and II are quite close but it seems that the former is more stable at higher temperatures.

Polymorph III presents a diagram with two clear endothermic peaks at 100 and 167°C. The existence of a desolvation process was dismissed because no loss of weight was detected by TGA. The heating of form III at 100°C resulted in the formation of a new polymorphic form (IV) which had a characteristic diffraction pattern (Fig. 2), so the DSC signal at 100°C is probably due to a polymorphic transition and the endothermic at 167°C can be assigned to the melting of polymorph IV.

The DSC profile of amorphous glisentide exhibits a very broad exothermic peak which is associated to a release of energy due to a crystallization process which results in the formation of polymorph IV and the broad endothermic peak at 164°C corresponds to its melting.

The DSC thermograms of the solvates with carbon tetrachloride and dioxane present broad endothermic peaks at temperatures near the boiling points of their respective solvents that can be assigned to desolvation processes, as it has been confirmed by TGA.

The loss of dioxane leads to the formation of polymorph II and this process can be easily monitored by X-ray diffraction analysis because there

Table 1
X-ray diffraction patterns of the main peaks for glisentide polymorphs and solvates with dioxane and carbon tetrachloride[a]

Form I		Form II		Form III		Form IV		Solvate dioxane		Solvate carbon tetrachloride	
°2θ	I	°2θ	I	°2θ	I	°2θ	I	°2θ	I	°2θ	I
4.9	m	10.7	w	9.3	w	12.0	m	7.6	h	5.9	w
9.8	h	11.7	h	10.5	w	15.0	m	13.6	w	11.7	m
11.8	w	12.7	w	12.0	w	17.2	w	14.3	w	14.1	w
12.7	w	13.4	m	13.3	m	19.0	h	15.3	w	15.0	w
15.9	m	14.4	w	16.7	w	19.8	w	17.1	w	15.5	w
16.5	w	17.5	w	18.0	w	21.3	w	18.0	w	16.4	w
16.9	m	18.2	w	20.3	h	22.0	h	19.9	h	16.7	w
17.3	w	18.7	m	21.2	w	24.4	h	22.1	w	17.6	m
18.3	w	20.0	m	23.4	w	26.1	w	23.0	w	17.9	m
18.5	w	20.7	w	23.9	w			24.5	w	19.4	h
20.5	h	21.8	h	24.8	w			25.3	w	20.0	w
21.4	w	23.0	m	27.2	w					20.7	w
22.2	h	25.0	h							22.0	w
22.9	w									23.3	h
24.3	w									26.4	w
24.9	m										
25.6	w										
26.0	w										

[a] Intensity (I); h, high ($I > 0.7I_{max}$); m, medium ($0.70I_{max} > I > 0.35I_{max}$); w, weak ($I < 0.35I_{max}$).

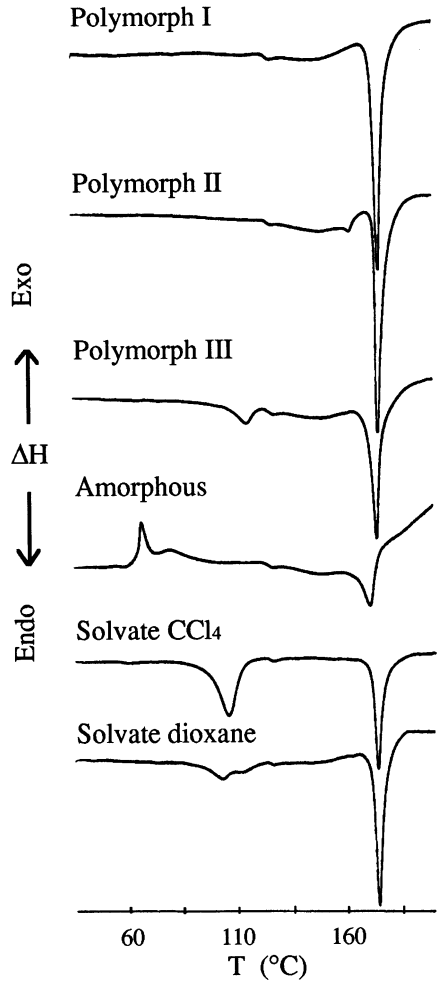

Fig. 3. DSC curves of the solid phases of glisentide.

is a progressive disappearance of the characteristic signals of the solvate as the reflections of form II appear in the diffraction profile. On the other hand, the loss of carbon tetrachloride gives rise to the formation of polymorph IV.

3.3. Thermogravimetric analysis

Thermogravimetric analysis has been used to confirm that the endothermic signals which appear in the DSC profiles of the solvates with dioxane and carbon tetrachloride correspond to a loss of solvent and also to evidence that there is no loss of weight associated to the endothermic peak present in the DSC curve of polymorph III. Table 2 shows the TGA data obtained for glisentide solvates, the loss of weight corresponds to a 1:1 molar stoichiometry in both solvates.

Table 2
TGA data for the solvates of glisentide with dioxane and carbon tetrachloride

Solvate	Weight loss found (%)	Weight loss expected for 1:1 stoichiometry (%)
Carbon tetrachloride	28.1	25.7
Dioxane	16.8	16.5

The loss of weight in the solvate with carbon tetrachloride takes place between 70 and 110°C and the boiling point of the solvent is 76.5°C, however, the loss of dioxane occurs between 40 and 120°C and the boiling point of dioxane is 101°C. The fact that the desolvation of dioxane starts at low temperatures might be related to the size and geometry of this molecule which does not allow to a close fitting within the crystal lattice of the solvate.

3.4. IR spectroscopy

There are considerable differences in the IR spectra of polymorphs I and II (Fig. 4); the rest of

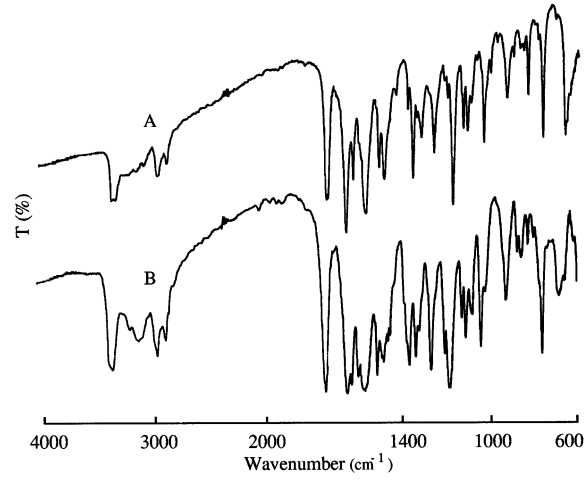

Fig. 4. IR spectra of polymorphs I (A) and II (B) of glisentide.

solid phases present IR patterns of one of these forms or a mixture of both. The assignment of bands in the IR spectrum of glisentide has been made according to the study carried out by Girgis-Takla and Dakas (1989). The main differences detected in the IR spectra of polymorphs I and II can be summarized as follows:

- the urea–NH band appears at 3310 and 3315 cm^{-1} in forms I and II, respectively, moreover, form II exhibits an additional band at 3100 cm^{-1}.
- the band at 1720 cm^{-1}, which corresponds to the aromatic carbonyl group, is present in both spectra, but it is broadened in that of polymorph II.
- the two bands attributed to the urea carbonyl group exhibit differences in position, relative intensities and broadening. These bands appear at 1635, and 1545 cm^{-1} in polymorph I and at 1620 and 1545 cm^{-1} in form II. In addition, the shoulder that is present in both cases is more intense in the spectrum of form II.
- the S=O stretching band is present at 1157 cm^{-1} in the spectrum of form I, but in that of polymorph II it shifts to 1165 cm^{-1}, it is broadened and it exhibits an intense shoulder at 1185 cm^{-1}.

On the basis of the study carried out by Girgis-Takla and Dakas (1989), it was initially thought that forms I and II of glisentide might have been related to a keto–enol tautomerism, because these authors reported similar modifications in the IR spectra of two polymorphic forms of acetohexamide and they proposed that both forms might have been tautomers. However, a more recent study (Stephenson et al., 1997) reported that both polymorphs of acetohexamide were in the keto form. Therefore, the polymorphism of glisentide is probably due to changes in crystal packing rather than conformational differences, as it has been observed to be the cause of polymorphism among most of the sulfonamide compounds (Byrn, 1982).

3.5. Optical microscopy

Optical microscopy has been used to characterize the crystalline habit of the different forms of glisentide. Polymorphs I and II are prisms which can be observed easily without magnification, however, forms III and IV give rise to aggregates of small crystals whose habits are difficult to define. The crystalline habit of the solvate with dioxane is very characteristic as it forms groupings of convergent tabular crystals which can be observed without magnification.

3.6. Stability of the different solid phases

The four polymorphic forms of glisentide are stable during 6 months at room temperature, however the amorphous phase of glisentide crystallizes within 3 weeks and both solvates experience desolvation processes.

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