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Investigation of the effects of grinding and co-grinding on physicochemical properties of glisentide

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Abstract

The purpose of the present study was to investigate the possibility of improving the dissolution properties of glisentide, a poorly water-soluble antidiabetic drug, by grinding in a high energy micromill, alone or in mixture with polyvinylpyrrolidone (PVP). Conventional and modulated differential scanning calorimetry (DSC, MDSC), thermogravimetry (TGA), X-ray powder diffraction (XRD), Fourier transform infrared spectroscopy (FT-IR), hot-stage FT-IR thermomicroscopy and scanning electron microscopy (SEM) were used to characterize the drug solid state, whereas its dissolution rates were determined according to the dispersed amount method. The techniques utilized enabled exclusion of polymorphism phenomena as a consequence of mechanical treatment, and revealed a progressive drug amorphization during grinding. In particular, MDSC allowed a clear determination of the glass transition temperature of the amorphous drug, enabling separation of glass transition from enthalpic relaxation. The amorphous state of the ground drug was the main responsible factor for the obtained 100% dissolution efficiency increase in comparison with the untreated drug. Further significant increases in dissolution properties, directly related to the polymer content in the mixture, were obtained by co-grinding with PVP, whose presence clearly favored drug amorphization, allowing a strong reduction of time and frequency of grinding necessary for obtaining complete drug amorphization.

Keywords: Glisentide; Grinding; Co-grinding; Dissolution; DSC; MDSC; FT-IR; X-ray powder diffraction

1. Introduction

Glisentide (GLI) ($C_{22}H_{27}O_5N_3S$), *N*-[2-[4-[[[(cyclopentylamino)carbonyl]amino]sulphonyl]phenyl] ethyl]-2-methoxy-benzamide is an oral hypoglycaemic agent, belonging to the sulphonylurea group, used in the therapy of type II non-insulin-dependent diabetes mellitus. Sulphonylureas show poor water solubility and dissolution properties and this can give rise to low and erratic bioavailability and poor dose proportionality [1,2]. The necessity to improve the dissolution properties of GLI has been suggested [3].

Grinding of poorly water-soluble drugs, alone or in the presence of suitable pharmaceutical adjuvants is a possible approach to this aim [4,5].

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Particle size reduction [6–8], decrease in drug crystallinity till amorphization [9,10], or formation of metastable polymorphic modifications [11,12] are possible factors responsible for the apparent increase in dissolution rate. These factors are of particular concern for GLI, which is reported to occur as polymorphic modifications as well as in a glassy state [13]. In addition, the use of the amorphous state or a metastable polymorph of a drug in pharmaceutical formulations gives rise to physical and/or chemical stability problems because the high-energy state of the drug can revert to the thermodynamically stable form or decompose more rapidly than the stable form under processing and storage conditions.

In the present work, we explored the possibility of improving GLI dissolution properties by grinding in a high energy micromill under carefully controlled conditions of frequency and time of mechanical treatment. Grinding was also performed on mixtures of GLI and polyvinylpyrrolidone (PVP) at various drug:adjuvant ratios in order to examine the effect of the polymer as both stabilizing agent of the solid-state of the drug and dissolution rate enhancer. Standard and modulated differential scanning calorimetry (DSC, MDSC), thermogravimetry (TGA) and simultaneous TGA/DSC, X-ray powder diffraction (XRD), Fourier transform infrared spectroscopy (FT-IR), hot-stage FT-IR thermomicroscopy and scanning electron microscopy (SEM) were used to characterize the solid state of the drug and polymer and to follow the phase transitions induced by mechanical treatments, heat and humidity exposition and ageing. The dissolution rates of drug alone and some drug–adjuvant combinations as powders (dispersed amount method) were determined and the results, in terms of dissolution efficiency (D.E.) and percent dissolved after 60 min, are discussed.

2. Materials and methods

2.1. Materials

GLI was a gift from J. Uriach, S.A. (Barcellona, E). PVP K30 , molecular weight 40,000, was supplied by Sigma Chemical Co. (St. Louis, USA). Organic solvents used for crystallization (ethanol, methanol, isopropanol) were of analytical reagent grade.

2.2. Preparation of crystal forms

A saturated solution of GLI in ethanol at 70 °C was cooled at room temperature. This same form was also obtained using methanol and isopropanol and corresponded to the form provided by the manufacturer and identified as the stable form I [13].

2.3. Preparation of ground samples

Samples of pure GLI were ground in a high energy vibrational mill (Mixer Mill Type MM 200, Retsch, GmbH, Düsseldorf, Germany) for different times (ranging from 15 to 180 min) and at two different vibration frequencies (15 and 30 Hz). Grinding jars (volume 12 cm^3) and stainless steel balls (9 and 12 mm diameter) were used. The total weight of each sample was about 1 g. No decomposition during treatment of the sample was observed by TLC.

2.4. Preparation of binary systems with PVP

Physical mixtures were prepared by 15 min of tumble mixing the 75–150 μm sieve granulometric fractions of the respective components at different drug–polymer (w/w) ratios (1:1, 1:3 and 3:1). Co-ground systems were prepared by grinding the corresponding physical mixtures in the high energy vibrational mill for different times (ranging from 5 to 30 min) at a vibration frequency of 15 Hz. No decomposition during treatment of the sample was observed by TLC.

2.5. Differential scanning calorimetry and modulated DSC

Conventional DSC and MDSC experiments were conducted using a Q 1000 DSC (Q™ series) (TA Instruments) equipped with a Tzero cell and a refrigerating cooling system and utilizing the advanced Tzero™ technology. Weighed samples

(2–3 mg, Mettler M3 microbalance) were scanned in covered aluminium pans under dry nitrogen purge (50 ml min^{-1}) at different heating rates over a temperature range of 30–190 °C. MDSC experiments were performed with a modulation amplitude of ± 2 °C and a 30 s modulation period with a 3 °C min^{-1} underlying heating rate. The instrument was calibrated for temperature using indium. The heat flow and heat capacity signals were calibrated using a standard sapphire sample. The Universal Analysis 2000 software was used to calculate extrapolated onset temperature, peak temperature and enthalpy value for each thermal event.

2.6. Thermogravimetric analysis and simultaneous TGA–DSC (STA)

TGA was carried out with a Mettler TA 4000 apparatus equipped with a TG 50 cell on 7–10 mg samples in open alumina crucibles over the 30–200 °C temperature range at the heating rate of 10 K min^{-1} under static air. Simultaneous recording of mass loss and heat flow was performed using a TGA/DSC Polymer STA 625 instrument (Polymer Laboratories, UK). Approximately, 5 mg of sample were scanned at 10 K min^{-1} from 24 to 200 °C under dry nitrogen flux (45 ml min^{-1}).

2.7. X-ray powder diffraction

XRD patterns were obtained with a Philips PW 1130 diffractometer (Co Kα radiation), at a scan rate of 2° min^{-1} over the 10–40 2θ range.

2.8. Fourier transform infrared spectroscopy

Spectra were recorded on a Perkin–Elmer Mod. 1600 FT-IR spectrophotometer, equipped with a deuterium triglycine sulfate (DTGS) detector. Spectra were obtained in the transmission mode in KBr pellets in the 4000–450 cm^{-1} region at 32 scans per spectrum.

2.9. Hot stage FT-IR thermomicroscopy

Analyses were performed with a Perkin–Elmer FT-IR microscope i-Series 2000 equipped with a mercury cadmium telluride (MCT) detector and Infrared Microspectroscopy Automated Graphical Environment (IMAGE) software, in the 4000–700 cm^{-1} region. Programmed sample heating was performed with a Micro-Press-H™ (Watlow) sample compression hot stage for infrared microscopes equipped with heater and thermocouple. Spectra were acquired in the reflection mode using an aluminium open inert support both at room temperature (25 °C) and during programmed heating (20 °C min^{-1} in the 30–150 °C range).

2.10. Scanning electron microscopy

SEM analysis was carried out using a Philips XL-30 SEM. Prior to examination, samples were gold-sputter coated to render them electrically conductive.

2.11. Dissolution tests

Dissolution tests were performed according to the USP 24 paddle method, using a Sotax AT7 apparatus (Sotax AG, Switzerland), by adding 5 mg of GLI or its equivalent in physical or co-ground mixture with PVP (60–100 μm granulometric sieve fraction collected before each experiment) to 1000 ml of artificial gastric medium without enzymes at 37 ± 0.3 °C, with constant stirring at 50 rpm. At suitable time intervals, aliquots were withdrawn and spectrophotometrically assayed for drug content at 288 nm (spectrophotometer Perkin–Elmer Lambda 2). Each test was performed in triplicate (coefficient of variation C.V. $< 3\%$). D.E. was calculated from the area under the dissolution curve at time t (measured using the trapezoidal rule) and expressed as a percentage of the area of the rectangle described by 100% dissolution in the same time [14].

3. Results and discussion

3.1. Solid state characterization of GLI and ground GLI

Representative powder X-ray diffraction patterns of GLI samples obtained by grinding at 15 or

30 Hz for different times are shown in Fig. 1. The X-ray spectrum of the untreated drug presents numerous and sharp reflections indicative of its high degree of crystallinity. The intensities of X-ray diffraction peaks decreased with increased grinding time and this effect was more marked at the highest grinding frequency. Increased grinding time caused a progressive change of the crystalline state of the drug into an amorphous state and an almost halo pattern was obtained after 180 min grinding at 15 Hz or 120 min grinding at 30 Hz.

The DSC curves of intact GLI and GLI samples obtained after various grinding times at a vibration frequency of 15 or 30 Hz are shown in Figs. 2 and 3, respectively. The thermal behavior of intact commercial GLI is identical to that of GLI samples obtained by recrystallization from ethanol, methanol or isopropanol and is characterized by a sharp endothermal effect

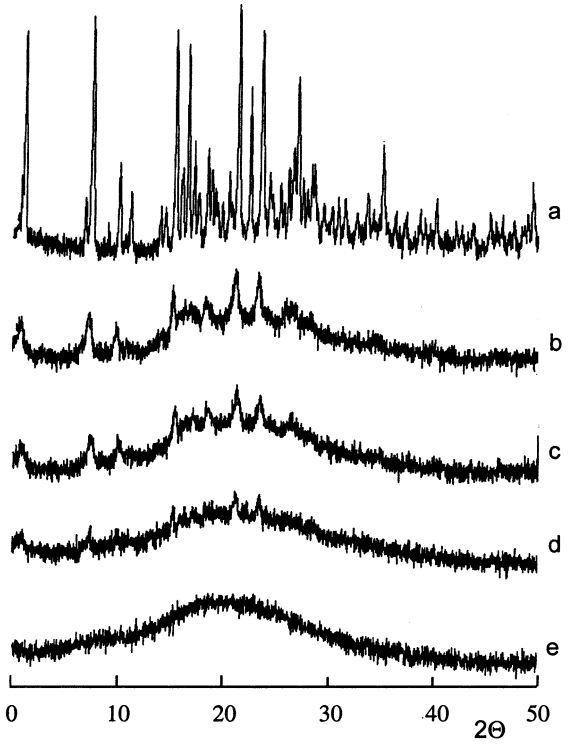

Fig. 1. Changes in powder X-ray diffraction patterns of GLI by grinding: (a) intact drug; (b) 30 min ground at 15 Hz; (c) 60 min ground at 15 Hz; (d) 60 min ground at 30 Hz; (e) 120 min ground at 30 Hz.

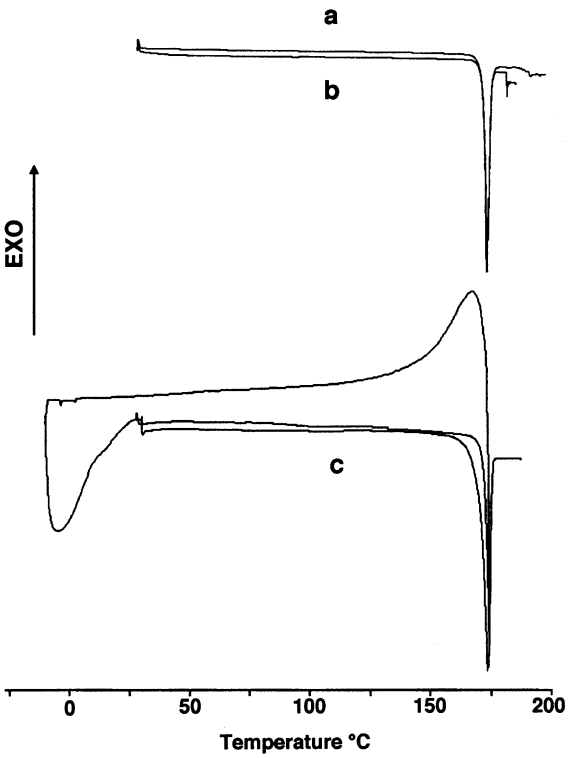

Fig. 2. DSC curves of GLI: (a) intact sample; (b) same sample after 7 days storage at 75% R.H.; (c) second run of the intact sample cooled quickly after first run (Instrument TA Q1000).

($T_{onset} = 172.3 \pm 1.5$ °C, $T_{peak} = 174.2 \pm 0.5$ °C, fusion enthalpy $= 84.8 \pm 2.6$ J g^{-1}, four runs) due to melting of anhydrous crystals of the pure drug, corresponding to the stable polymorph form I [13]. No weight loss was detected by TGA. No variations in the drug DSC curve were observed after 7 days storage under 75% RH conditions at room temperature, nor in a second DSC run, recorded after heating a drug sample up to 175 °C and then quickly cooling it to 10 °C: in all cases only the melting peak of the stable polymorph I was observed (Fig. 2).

A small endothermic peak appeared at about 52 °C after 30 min of sample grinding at 15 Hz, followed by a broad exothermic effect peaked at about 64 °C and then by the melting peak of the stable form I at 173.2 °C (Fig. 3A). The first endothermic peak remained almost unchanged with increasing grinding time, whereas a progressive shift of the exothermic effect at higher

temperatures (up to 84 °C after 180 min grinding) was observed, with a concomitant increase of the enthalpic variation (from 8.3 up to 34.3 J g^{-1}). These same thermal effects appeared after shorter times (only 15 min) at the highest frequency vibration (30 Hz) and the exothermic band was more intense and markedly shifted at higher temperatures, occurring at about 130 °C after 120 min grinding (Fig. 3B). The results suggest that the ground GLI may be in the glassy state and changes to polymorph melting at 173 °C during non-isothermal heating. The broad exothermic peak was associated to a release of energy due to a recrystallization process of the amorphous GLI resulting in the formation of the stable polymorph I. It was proposed that the higher energy states produced by grinding progressively converted the crystalline structure of GLI into an amorphous state, since, as the grinding time increased, the exothermic peak due to recrystallization was observed as becoming clearer, more intense and it shifted to higher temperatures. In the case of grinding at 30 Hz, no further variations of the drug thermal curve were observed when the grinding time was increased from 120 to 180 min, indicating that drug amorphization was already complete after 120 min of mechanical treatment. In any case, irrespective of the time and frequency of grinding treatment, glassy GLI always recrystallized during the DSC scan into the original crystalline form I. The small endothermic effect at about 60 °C, which had no associated mass loss as proved by STA, was attributed to a glass transition because of the increase in heat capacity of GLI. A similar DSC behavior, before the exothermic band of recrystallization, has been observed also for the thermal curve of glassy glibenclamide obtained by quick cooling of the crystalline form [15]. A glass transition temperature of 56.2 ± 0.7 °C (three replicates) was determined by taking the integral of the power/temperature plot and measuring the intercept of the extrapolated glass and liquid enthalpy lines according to Richardson's method [16]. The glass transition temperature was close to that reported for nifedipine [17] and tolnaftate [18] and the ratio between this temperature and melting point of 0.75 was within the range reported so far for other glassy compounds [18]. The enthalpy values for the relaxation endotherm calculated by the construction of various types of baselines beneath the DSC peak were 6.7 J g^{-1} (sig-horizontal baseline), 5.5 (linear baseline) and 4.7 g^{-1} (sig-tangent baseline).

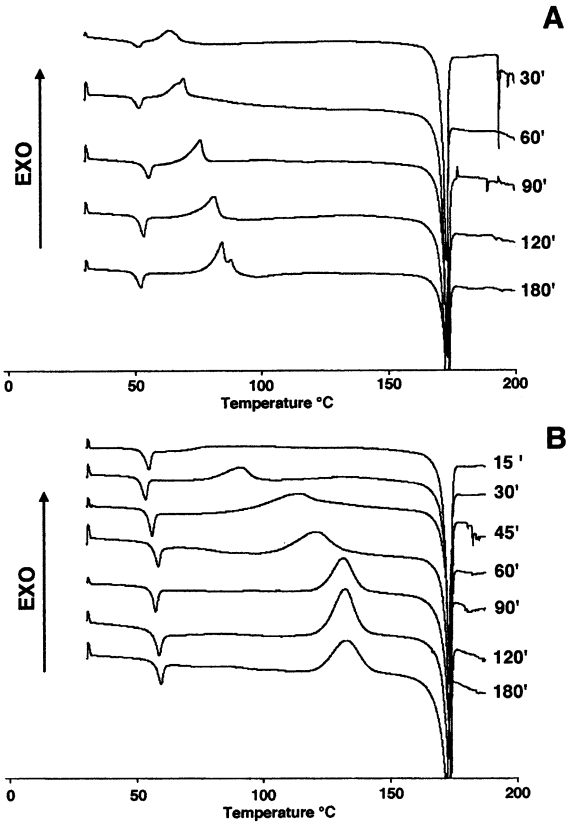

Fig. 3. Changes of DSC curves of GLI during grinding in a high energy ball-mill at 15 (A) or 30 Hz (B) (TA Q1000 Instrument).

Fig. 4 represents the MDSC response of the glassy GLI sample. This technique, involving the application of a sinusoidal heating signal superimposed on the linear program, allowed separation of the total heat flow signal of conventional DSC into the reversing and non-reversing signals. A glass transition at 62.6 °C may be seen in the reversing heat flow signal, while an onset temperature of 55.6 °C and a relaxation enthalpy of 7.4 g^{-1} (horizontal baseline) were de-

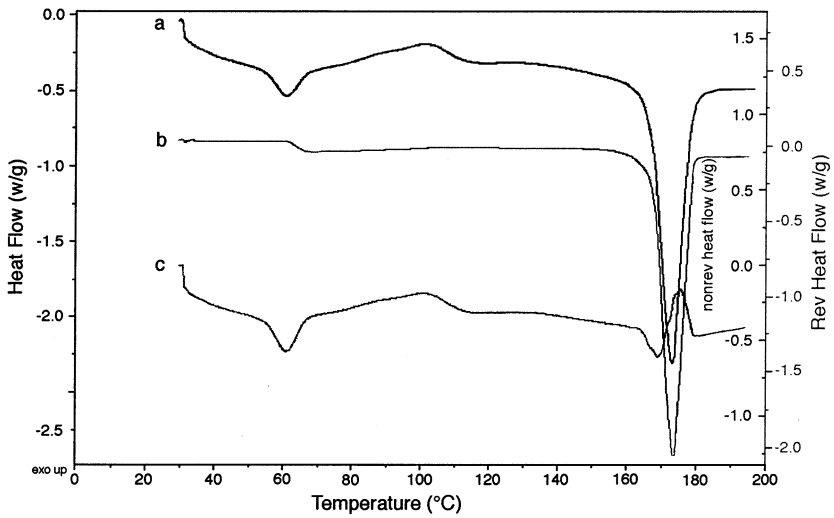

Fig. 4. MDSC curve of ground GLI (120 min at 30 Hz) with separation of the total DSC response (curve a) into the reversing (curve b) and non-reversing (curve c) heat flow signals (TA Q1000 Instrument).

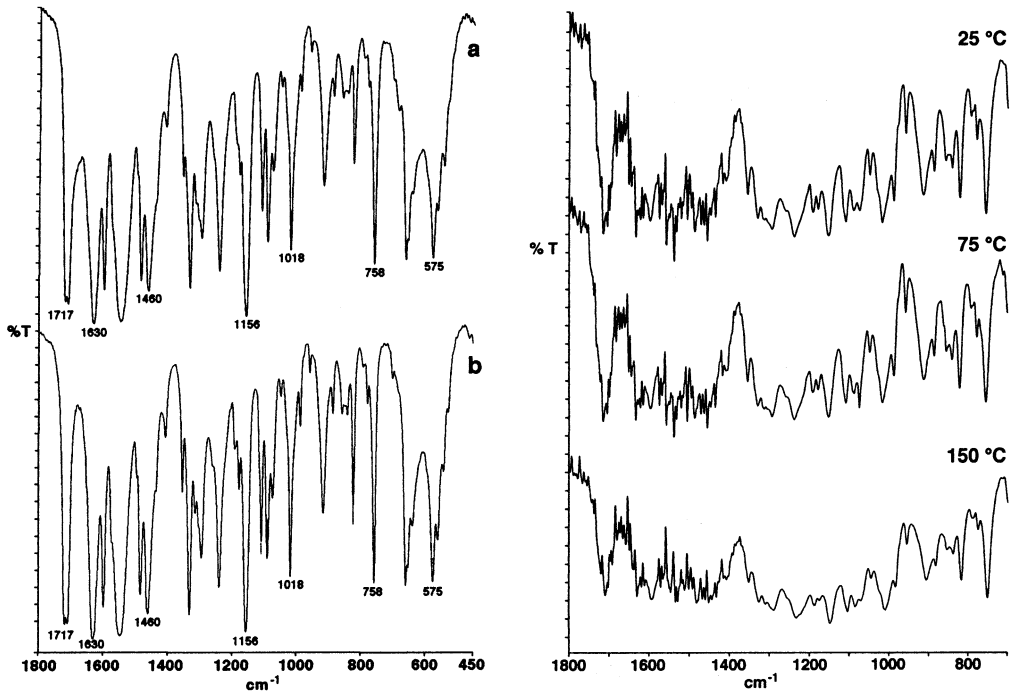

Fig. 5. FT-IR spectra of GLI: (a) Intact commercial sample; (b) the same sample after 90 min heating at 80 °C; on the right: Hot-stage FT-IR spectra of GLI intact commercial sample at room temperature and heated up to 150 °C (heating rate 20 °C min^{-1}).

termined for the relaxation endotherm observed in the non-reversing signal.

The DSC curve of amorphous ground GLI appeared almost unchanged after 4 months storage at room temperature in a closed glass container, indicating the stability of the glassy state, whose chemical stability was also confirmed by TLC analysis which did not show any evident decomposition phenomenon.

The FT-IR spectrum of commercial GLI, recorded in KBr pellets (Fig. 5a) was identical to those of GLI recrystallized from ethanol, methanol and isopropanol, and corresponded to that previously reported for the polymorph I [13]. The most interesting bands are in the zone between 1800 and 1100 cm^{-1} and, in particular, at 1717 cm^{-1}, corresponding to the aromatic carbonyl group, at 1630 cm^{-1}, attributed to the urea carbonyl group, and at 1156 cm^{-1}, due to the S=O stretching band. No changes were observed in the commercial GLI spectrum recorded after 90 min sample heating at 80 °C (Fig. 5b). Heating effects were better studied by hot-stage FT-IR thermomicroscopy. A typical spectrum of commercial GLI recorded in the reflection mode at room temperature (25 °C) is shown in Fig. 5. The sample was then heated at 20 °C min^{-1} up to 150 °C. All spectra recorded up to this final temperature were similar to the spectrum at room temperature, indicating that no phase modifications occurred as a consequence of heating.

FT-IR spectra of GLI ground at 30 Hz for 120 min, recorded both in the transmission mode in KBr pellets, and in the reflection mode in an aluminium open inert support, were compared with the corresponding ones obtained for the intact sample (Fig. 6) to evaluate the possible effects of the increased internal energy of the sample as a consequence of the mechanical treatment. No distinctive changes were observed for

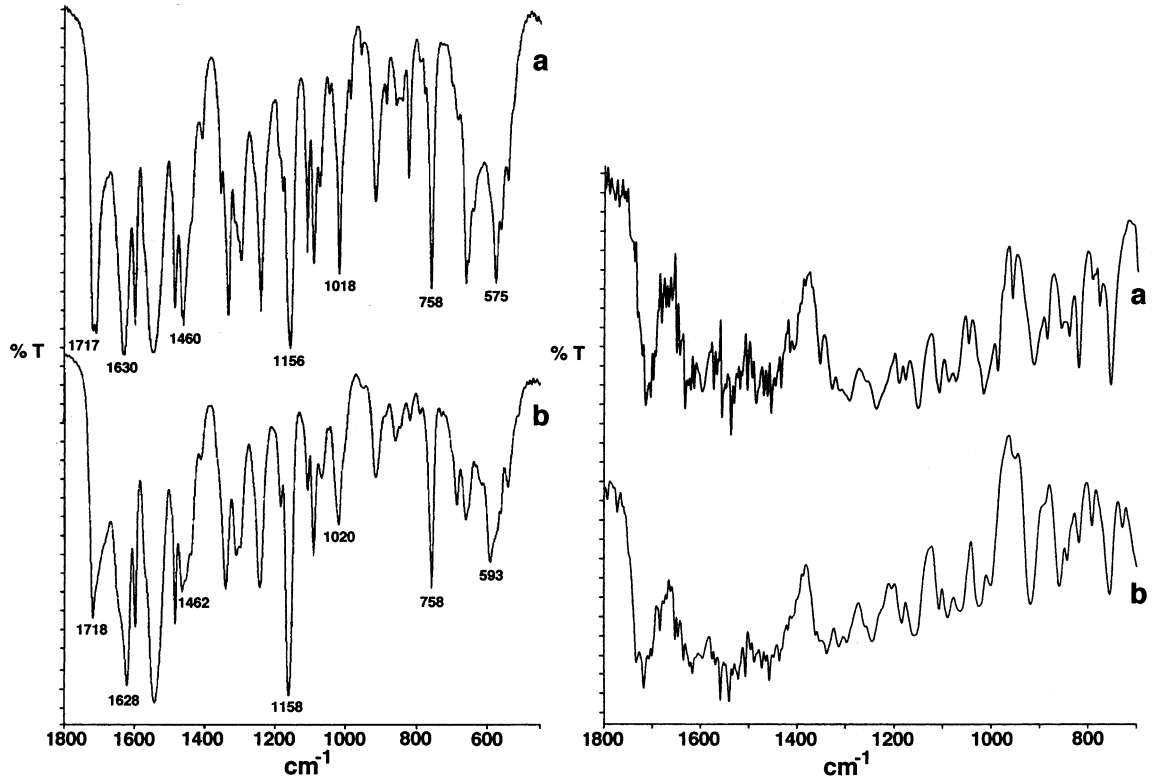

Fig. 6. FT-IR spectra (left, transmission mode) and hot-stage FT-IR spectra (right, reflection mode) of GLI: (a) intact commercial sample; (b) ground sample (120 min at 30 Hz).

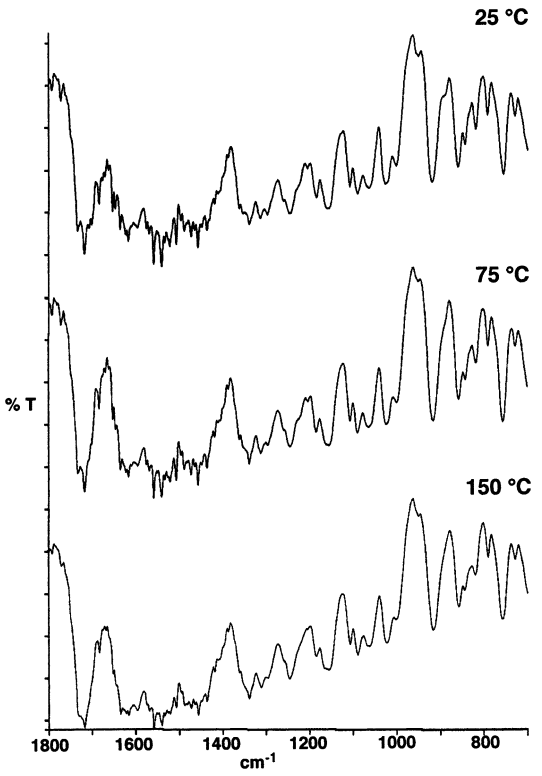

Fig. 7. Hot-stage FT-IR spectra of GLI ground sample (120 min at 30 Hz) at room temperature and heated up to 150 °C (heating rate 20 °C min^{-1}).

the principal GLI spectral bands, indicating that no polymorphic transitions appear to have occurred. On the contrary, a general reduction of intensity of the bands and a loss of spectral resolution were observed, both effects attributable to the drug amorphization process. No heating effects were detectable on spectra recorded by the hot-stage FT-IR thermomicroscope on the GLI ground sample heated at 20 °C min^{-1} up to 150 °C, indicating the thermal stability of the amorphous ground sample (Fig. 7).

Commercial GLI powder appeared under the SEM as formed by typical polyhedric crystals of variable dimensions, ranging from 60 to 100 μm. Microscopic examination revealed striking differences in the morphology of the ground GLI with respect to the commercial one. In fact the ground GLI appeared as particles of irregular forms characterized by a rough and porous surface, thus revealing its amorphous nature (Fig. 8).

3.2. Solid state characterization of GLI–PVP coground systems

Further studies were carried out in order to investigate the effect of the presence of an amorphous excipient as PVP K30 on the GLI amorphization process under grinding.

Fig. 9 shows representative powder X-ray diffraction patterns of GLI–PVP physical and coground mixtures. The characteristic diffraction peaks of GLI crystals were clearly detected in all the physical mixtures, emerging from the halo pattern of the amorphous PVP, whereas they considerably decreased in the corresponding coground mixtures. This effect was more marked for

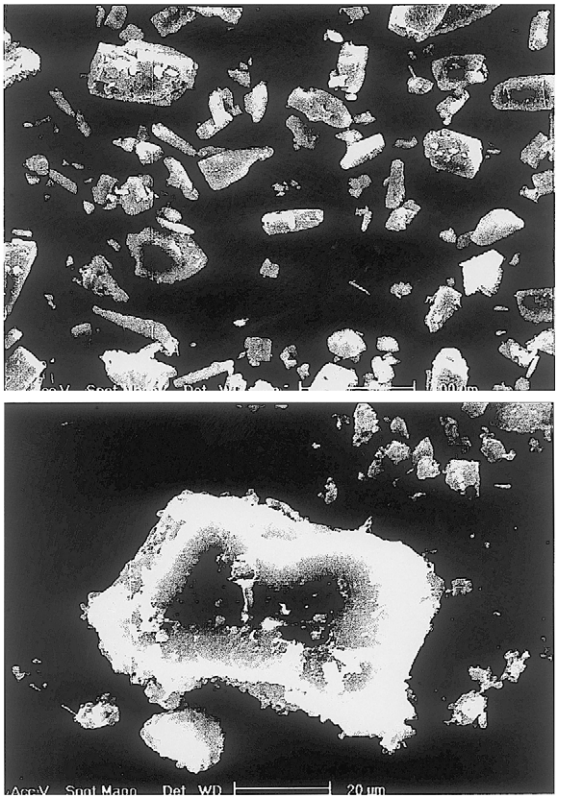

Fig. 8. SEM microphotographs of GLI: intact commercial sample (top) and ground sample (120 min at 30 Hz) (bottom).

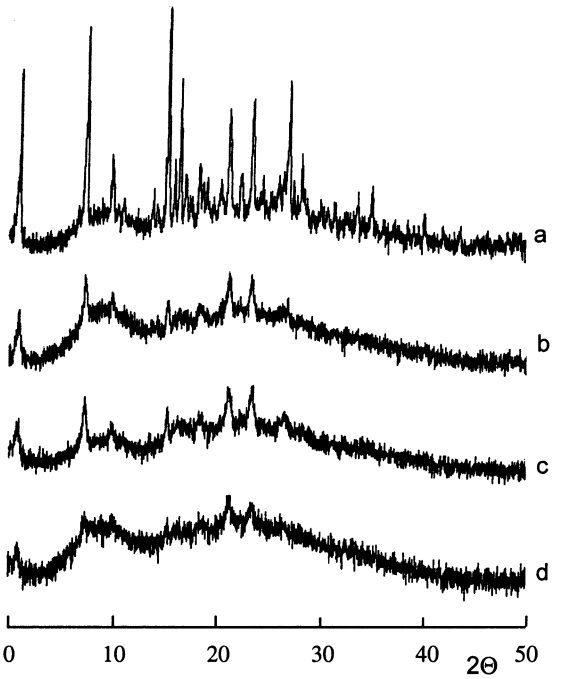

Fig. 9. Representative powder X-ray diffraction patterns of GLI–PVP mixtures: (a) 1:1 (w/w) physical mixture; (b) 1:3 (w/w) co-ground mix (5 min at 15 Hz); (c) 1:1 (w/w) co-ground mix (20 min at 15 Hz); (d) 1:3 (w/w) co-ground mix (20 min at 15 Hz).

combinations at higher PVP content. Almost complete drug amorphization was observed for the 1:3 (w/w) GLI–PVP systems after only 30 min co-grinding at the lowest frequency (15 Hz).

Fig. 10 shows the DSC curves of GLI–PVP binary mixtures at different (w/w) ratios (3:1, 1:1 and 1:3) after different grinding times (0–30 min) at a vibrating frequency of 15 Hz. The thermal behavior of PVP was that expected for hygroscopic, amorphous substances, with a large endothermal effect in the 50–110 °C temperature range due to polymer dehydration (water content 9% w/w by TGA). The weight loss (recorded by TGA) in the different drug–polymer combinations examined was in all cases proportional to the amount of PVP in the mixture. The thermal curves of the physical mixtures were the simple superimposition of those of the single components, even though some broadening and reduction of intensity of the drug melting peak were observed, particularly in the 1:3 (w/w) drug–PVP system. A sharp decrease of drug endothermic melting peak, accompanied by broadening and shifting to lower temperatures, was observed for all samples after only 5 min grinding. This effect was not greatly influenced by the grinding time, whereas it depended markedly on the PVP content in the mixture. Almost complete disappearance of the drug melting peak, indicative of its amorphization, was observed in the 1:3 (w/w) GLI–PVP 30 min co-ground system, in agreement with the X-ray analysis results. DSC and

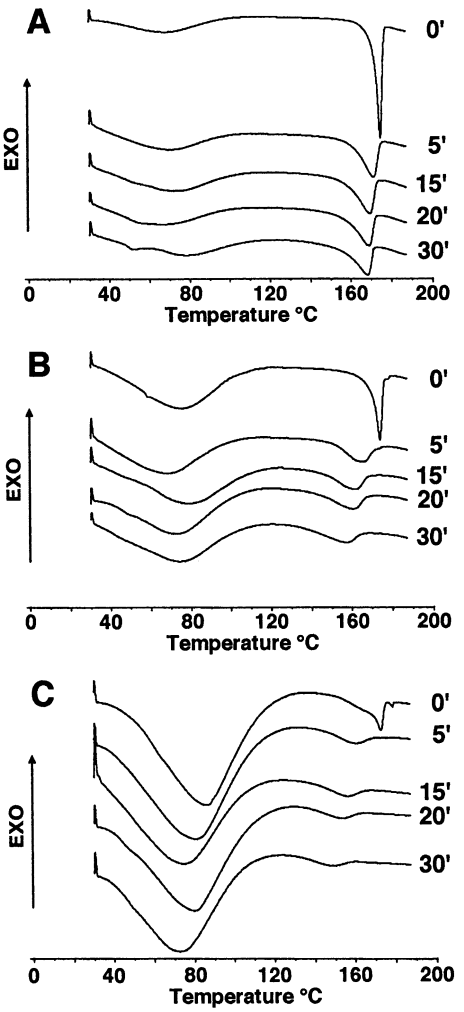

Fig. 10. Changes of DSC curves of GLI–PVP mixtures at different w/w ratios ((A) 3:1; (B) 1:1; (C) 1:3) during co-grinding (frequency 15 Hz).

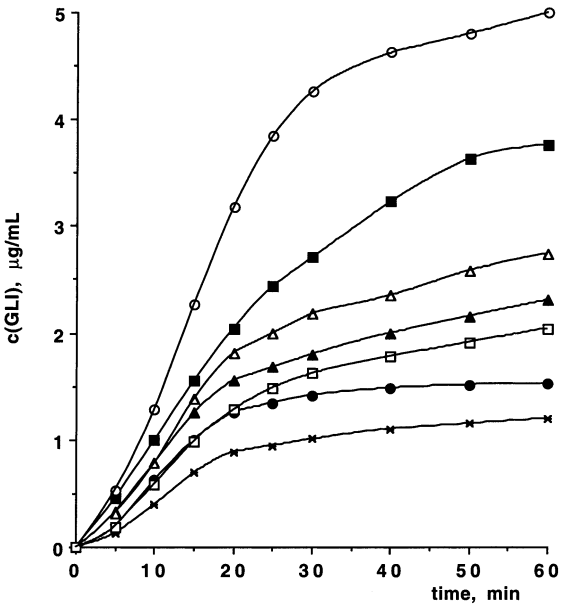

Fig. 11. Dissolution curves of GLI and GLI–PVP physical (P.M.) and co-ground (GR) mixtures at different w/w ratios. Key: (★) intact GLI; (□) ground GLI (60 min 30 Hz); (▲) ground GLI (120 min 30 Hz); (●) GLI–PVP 1:3 P.M.; (△) GLI–PVP 1:1 GR (30 min 15 Hz); (■) GLI–PVP 1:3 GR (15 min 15 Hz); (○) GLI–PVP 1:3 GR (30 min 15 Hz).

TLC analyses of 4-month aged drug–polymer co-ground samples (room temperature in a closed glass container) allowed assessment of their physical and chemical stability.

3.3. Dissolution tests

The dissolution curves of GLI, untreated or ground (60 or 120 min at 30 Hz), and of some representative GLI–PVP systems are shown in Fig. 11. As can be noted, grinding allowed a significant improvement of the dissolution properties of the drug, and this effect was more evident with increasing the intensity of mechanical treatment (in terms of frequency and duration of grinding). Both percent dissolved and D.E. at 60 min of the 120 min ground GLI sample (46 and 31%, respectively) were about twice in comparison to those of the untreated drug (24 and 16%, respectively). This finding is reasonably due to both particle size reduction and the progressive drug amorphization process during the mechanical treatment, as confirmed by X-ray and DSC analyses. Further increases of GLI dissolution rate were obtained by co-grinding the drug with amorphous PVP. The slight improvement of drug dissolution behavior observed for the simple physical mixtures could be attributed to the better wettability of the drug due to the presence of the hydrophilic polymer, which can reduce the interfacial tension between the poorly-soluble drug and the dissolution medium. The evident enhancement in the drug dissolution rate shown by co-ground mixtures may be attributed not only to the more intimate dispersion of GLI into the carrier, as a result of the mechanical treatment, but also to the amorphous or nearly amorphous state of such systems, as shown by DSC and X-ray diffraction analyses. This effect was more marked as the carrier content in the mixture increased. None of the products showed significant changes in their dissolution properties after 4 months ageing at room temperature.

4. Conclusions

Grinding of GLI in a high energy mill positively influenced its dissolution properties, allowing a 100% D.E. increase. The different techniques utilized to appropriately characterize the drug solid state properties enabled exclusion of possible polymorphism phenomena as a consequence of the drug mechanical treatment. On the contrary, a progressive drug amorphization process during grinding was shown, which was considered the main responsible factor for the bettering of GLI dissolution performance.

A further significant increase in drug dissolution properties was obtained by co-grinding with PVP. It was directly related to the mass fraction of polymer in the mixture: in particular, the 1:3 (w/w) GLI–PVP co-ground product was the only one which allowed achievement of 100% dissolved drug, with a D.E. of about 67%, i.e. more than twice in comparison to that of GLI ground alone.

Finally, it is important to remark that co-grinding in the presence of PVP clearly favored the drug amorphization process. In fact, at least 2 h of grinding at the higher frequency (30 Hz) were

required for obtaining complete amorphization of GLI alone, while the same transformation was brought about after half 1 h of grinding at the lower frequency (15 Hz) in the 1:3 (w/w) GLI–PVP mixture.

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