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SOLID-STATE CHARACTERIZATION OF GLYBURIDE-CYCLODEXTRIN CO-GROUND PRODUCTS

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Abstract

Natural crystalline (α-, β-, γ-) and amorphous derivative (hydroxypropyl-β- and methyl-β-) cyclodextrins were selected as potential carriers for obtaining, through a co-grinding technique, a stable activated amorphous form of glyburide with improved dissolution properties. Differential scanning calorimetry (DSC) was used to investigate solid-state modifications of the drug induced by co-grinding with the selected carriers in a high energy vibrational micro-mill. X-ray powder diffraction and FTIR spectroscopy were employed as additional techniques to support DSC data. Equimolar drug : cyclodextrin physical mixtures were co-ground for different times (up to 60 min) at constant vibration frequency (24 Hz). A progressive drug amorphization with increasing grinding time was observed in all binary systems, but, interestingly, different degrees of sensitivity to the mechanical-chemical activation were evident. In fact, blends with natural cyclodextrins, despite the initial higher crystallinity than those with the amorphous derivatives, required the same or shorter co-grinding times (60 min) to achieve complete drug amorphization. Stability studies indicated no appreciable drug recrystallization in co-ground products after 4 months storage in sealed containers at 25°C or 1 month at 25°C and 75% RH. No stability differences were detected between products with natural or derivative cyclodextrins. The results accounted for the suitability of cyclodextrin co-grinding technique to obtain and stabilize glyburide in the activated amorphous form.

Keywords: co-grinding, cyclodextrin, DSC, glyburide

Introduction

Glyburide (5-chloro-N-{2-[4-({[(cyclohexyl-amino)carbonyl]amino}sulfonyl)phenyl]ethyl}-2-methoxybenzamide) is an oral hypoglycemic agent, belonging to the second generation of sulfonylureas, whose poor aqueous solubility can give rise to variations in its dissolution rate and incomplete and largely variable bioavailability [1–2]. Over the last few years different approaches aimed at enhancing glyburide dissolution properties have been employed, such as solid dispersion methods [3–5] or complexation with cyclodextrins [6–8].

On the other hand, it is known that conversion of poorly water soluble crystalline drugs into the amorphous state is another possible approach for improving the

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biopharmaceutical properties of solid dosage forms [9]. Amorphization of glyburide by spray-drying has been tried, but the activated and more soluble amorphous form was highly unstable and rapidly reverted to the crystalline form [10]. Several works have shown that drug amorphization can be obtained by producing a molecular dispersion by grinding the drug with suitable pharmaceutical adjuvants, such as cellulose, chitin or chitosan, cyclodextrins, polyvinylpyrrolidone [11–14]. The extent of amorphization generally depends on both the type and the relative amount of the additive and the grinding time [15]. Moreover, the adjuvant can sometimes act as a stabilizing agent of the obtained amorphous state, by preventing or at least slowing down drug recrystallization [14, 16].

In the present work we tested the effectiveness of both natural crystalline (α-, β- and γ-) and amorphous derivative (hydroxypropyl-β- and methyl-β-) cyclodextrins as potential carriers for improving glyburide dissolution through drug amorphization obtained by co-grinding in a high energy vibrational micro-mill. Solid-state modifications of glyburide induced by co-grinding with the selected carriers under frequency and time-controlled conditions, were investigated by differential scanning calorimetry (DSC), since this technique proved to be a very powerful analytical tool in the study of drug–cyclodextrin interactions [17–19]. X-ray powder diffraction (XRPD) and Fourier transform infrared spectroscopy (FTIR) were also employed as additional techniques to support DSC results.

Moreover, since the amorphous forms are in general thermodynamically unstable and more reactive than their crystalline forms, it is important to evaluate their stability and the influence of ageing conditions on drug recrystallization [20]. Thus, samples of the amorphous systems obtained were stored under two different controlled conditions (four months in sealed containers at 25°C or one month at 25°C and 75% RH) and periodically tested by DSC analysis, in order to reveal any possible drug recrystallization processes occurring during storage.

Experimental

Materials

Micronized (2 μm) glyburide (GLY) was obtained from Guidotti Laboratori S.p.A. (Italy).

α-, γ- and methyl-β-cyclodextrins (α-Cd, γ-Cd, Meβ-Cd) were kindly supplied by Wacker Chemie GmbH (München, Germany), whereas β- and hydroxypropyl-β-cyclodextrins (β-Cd, HPβ-Cd) were donated by Roquette Italia S.p.A..

Solid systems preparation

Equimolar (1:1 mol/mol) physical mixtures of GLY and each Cd were prepared by gently mixing with a spatula in an agate mortar for 15 min of the previously sieved (75–250 μm sieve granulometric fraction) components. The homogeneity of the blends was checked by DSC measurements (see below) of three samples for each preparation. Co-ground systems were obtained by grinding the corresponding blends

in a high energy vibrational micro-mill (Mixer Mill Type MM 200, Retsch, GmbH, Düsseldorf, Germany) at a vibration frequency of 24 Hz for different times (15, 30, 45 and 60 min). Grinding jars of 25 cm^3 and stainless steel balls of 9 and 12 mm diameter were used. The total mass of each sample was about 500 mg.

DSC

Temperature and enthalpy measurements were performed with a Mettler TA4000 Star^e apparatus (Mettler Toledo, Switzerland) equipped with a DSC 25 cell. Samples of about 5–10 mg were weighed (Mettler M3 microbalance) in pierced Al pans and scanned under static air over a temperature range from 30 to 200°C at a heating rate of 10°C min^{-1}. Calibration of temperature and heat flow was performed with standard indium samples. The relative degree of crystallinity of GLY in physical and ground mixtures, expressed as a percentage of the GLY mass fraction in the starting sample, $GLY_{RDC\%}$, was calculated by Eq. (1) [19]:

$$GLY_{RDC\%}=100\frac{\Delta H_{mix}}{\Delta H_{st}} \quad (1)$$

where ΔH_{mix} and ΔH_{st} are the heats of fusion of GLY measured in the physical and ground mixtures and in the starting pure GLY sample, respectively. Heat of fusion measurements were carried out in duplicate and the relative standard deviation of crystallinity data was ±5%.

XRPD

XRPD patterns were obtained with a Philips PW 1130 powder diffractometer (CoK_α radiation), over the 10–50° 2θ range at a scan rate of 1° min^{-1}.

FTIR

FTIR spectra were recorded in the 4000–450 cm^{-1} spectral region on a Perkin Elmer Mod. 1600 apparatus using KBr pellets.

Stability studies

About 100 mg of selected co-ground samples were weighed in sealed glass vials and stored for four months at 25°C or placed in sealed desiccators containing saturated salt solutions equilibrated at 75% RH and 25°C and stored there for one month. At defined time intervals, aliquots were withdrawn and subjected to DSC analysis.

Results and discussion

Solid-state characterization

The thermal behaviour of pure drug and Cds and of their equimolar physical mixtures and co-ground products at different grinding times is reported in Figs 1 and 2. The results

of DSC studies in terms of drug melting temperatures and enthalpies and relative degree of crystallinity (RDC%), calculated by the decrease in GLY fusion enthalpy with respect to the starting drug (see Materials) are collected in Table 1. The DSC curve of pure GLY displayed a sharp endothermic effect peaking at 175.3±0.4°C with a fusion enthalpy of 95±3 J g^{-1}, associated with the melting process of the anhydrous crystalline drug. A typical three-step dehydration DSC pattern was shown by α-Cd, while the thermal profile of both β-Cd and γ-Cd exhibited a single sharp dehydration band peaking at 125 and 110°C, respectively. A broad endotherm ranging between 60 and 120–130°C and associated with water loss was shown by both Cd-derivatives.

Table 1 Effect of grinding on temperatures and enthalpies of GLY melting peak in its equimolar combinations with natural and derivative cyclodextrins, and relative degree of crystallinity (RDC) with respect to the drug alone

Binary systems	Grinding time/min	T_{fus}/°C	ΔH_{fus}/J g^{-1}	RDC/%
GLY-α-Cd	0	174.6	84.7	89
	15	169.5	48.7	51
	30	169.0	25.0	26
	45	168.3	13.2	14
	60	–	0	0
GLY-β-Cd	0	174.6	83.3	88
	15	170.3	62.0	65
	30	167.8	41.5	44
	45	169.2	18.9	20
	60	–	0	0
GLY-γ-Cd	0	175.0	85.3	90
	15	170.6	65.1	68
	30	167.9	39.1	41
	45	166.7	13.8	14
	60	166.2	1.4	1
GLY-HPβ-Cd	0	172.6	75.9	80
	15	166.1	62.8	66
	30	162.7	32.7	34
	45	160.8	10.5	11
	60	159.5	4.5	5

The melting endotherm of GLY was substantially unaffected in its shape, area and peak temperature by mixing with natural Cds, and hence, the drug maintained a high degree of crystallinity in such systems. By contrast, a broadening and shifting of drug melting peak was observed for blends with HPβ-Cd and even more so with Meβ-Cd,

accompanied in this latter case by a marked reduction in its intensity. All these phenomena were indicative of a significant decrease in GLY crystallinity as a consequence of mixing with the amorphous partners. The drug amorphization became more evident in co-ground products, due to both the particle size reduction and the more intimate physical contact between the components brought about by the mechanical treatment. The effect of grinding on drug amorphization increased with the grinding time, as shown by the progressive broadening and lowering of temperature of the GLY melting peak and the concomitant reduction of the related enthalpy. These findings accounted for the presence of marked interactions between drug and cyclodextrins induced by the co-grinding treatment and leading to drug amorphization

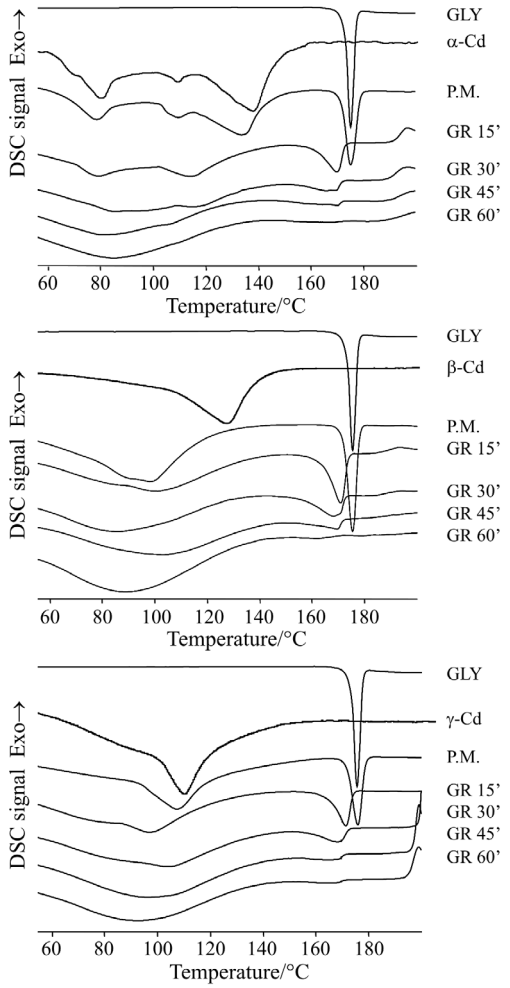

Fig. 1 DSC curves of pure GLY and natural cyclodextrins and their equimolar physical (P.M.) and co-ground (GR) mixtures after different grinding times

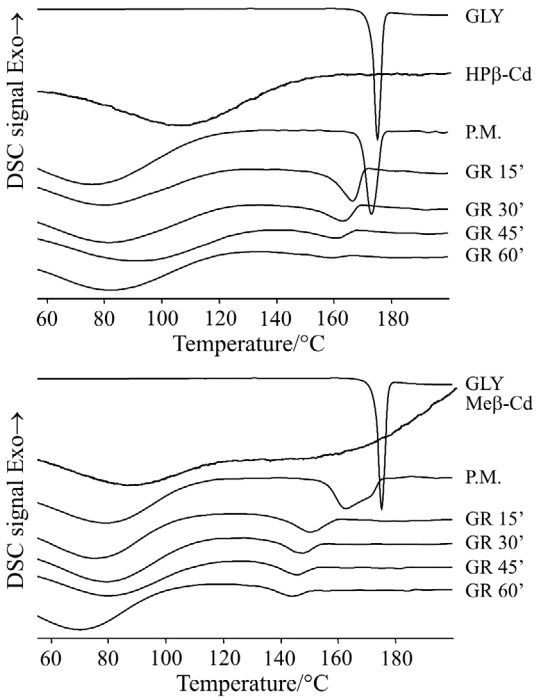

Fig. 2 DSC curves of pure GLY and derivative cyclodextrins and their equimolar physical (P.M.) and co-ground (GR) mixtures after different grinding times

and/or complexation [14]. However, unexpectedly, this effect was more marked in combinations with natural Cds. In fact, despite the high degree of drug crystallinity in their blends, complete amorphization, as indicated by the total disappearance of the GLY melting peak, was obtained after 60 min co-grinding. In contrast, a small and broad endothermic effect, attributable to the drug melting, and thus indicative of some residual drug crystallinity, was still detectable in 60 min co-ground products with both amorphous derivatives, in spite of the initial stronger amorphizing power of their blends with the drug. This unusual behavior was particularly evident in combinations with Meβ-Cd, which showed on the one hand the greatest drug crystallinity loss in physical mixture (RDC: 60%) and in the 15 min co-ground product (RDC: 27%), and on the other the least effect of improved drug amorphization with increasing grinding time (RDC about 10% after 60 min co-grinding).

XRPD spectra of pure single components and of some representative drug–Cd binary systems are shown in Fig. 3. The diffraction pattern of the drug exhibited numerous distinctive and sharp peaks, typical of a crystalline product. X-ray patterns of natural cyclodextrins indicated their crystalline nature, whereas both Cd-derivatives showed diffuse patterns, typical of amorphous substances. The spectra of physical mixtures with α-Cd and γ-Cd were almost the simple superimposition of those of their pure components, whereas some crystallinity loss can be appreciated in

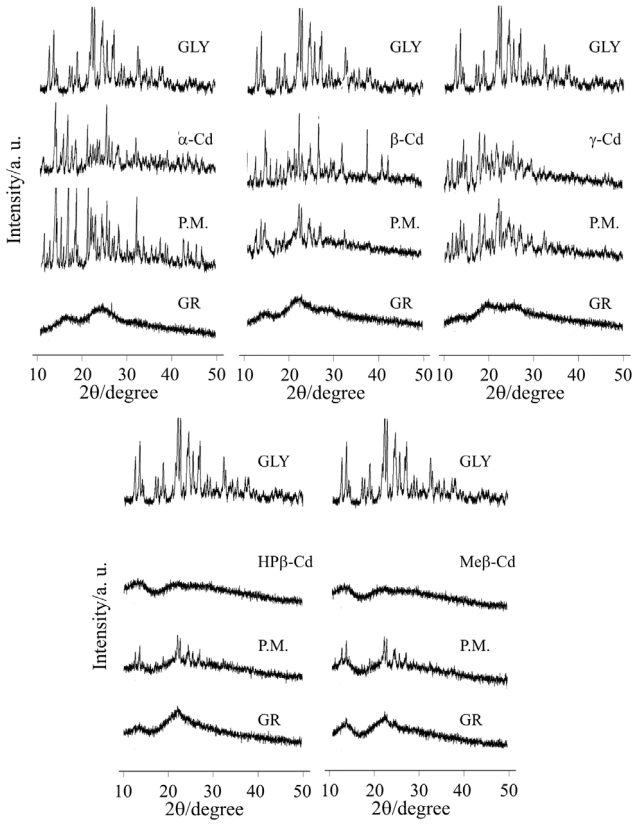

Fig. 3 X-ray powder diffraction patterns of pure components and their equimolar physical (P.M.) and co-ground (GR) mixtures after 60 min grinding

the blend with β-Cd. By contrast, a marked decrease of both number and peak intensities was observed in physical mixtures with both HPβ-Cd and Meβ-Cd. The differences observed between blends with natural or amorphous cyclodextrins were not more evident after grinding. In fact, after 60 min co-grinding, no significant drug residual crystallinity was still detectable and a diffuse pattern, indicative of the almost complete amorphous state of the samples, was observed for all the systems.

Drug–Cd solid-state interactions were also investigated by FTIR spectroscopy (Fig. 4). The spectrum of GLY showed characteristic absorption bands at 1715, 1617 and 1523 cm^{-1}, attributed to the amide and urea carbonyl stretching vibrations and to the urea N–H bending, respectively [21]. Different behaviours were evidenced among binary systems with natural and amorphous Cds. In fact, in the case of natural Cds, the patterns of physical mixtures were the simple superimposition of those of the single components, whereas those of the corresponding co-ground systems showed the almost complete disappearance of the band at 1715 and the concomitant shift to higher

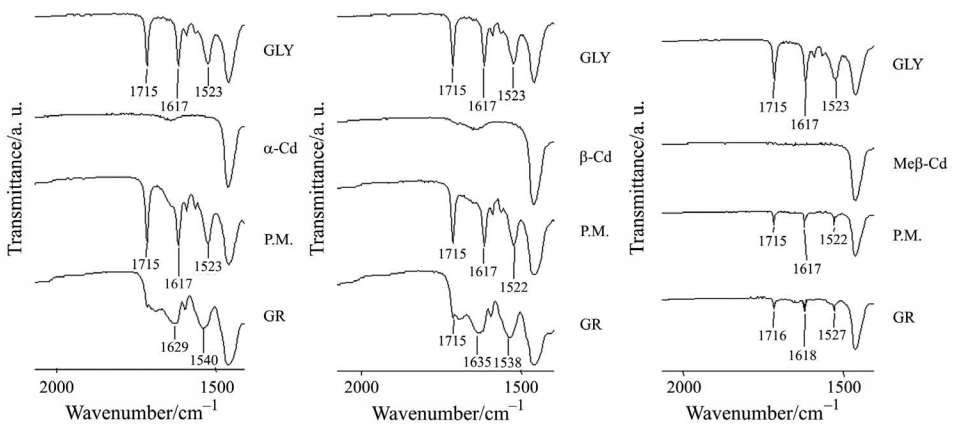

Fig. 4 FTIR spectra of pure components and their equimolar physical (P.M.) and co-ground (GR) mixtures after 60 min grinding

frequencies of the 1617 and 1523 bands, as a consequence of solid-state intermolecular interactions, such as hydrogen bonding formation, induced by mechanical treatment of the sample. In contrast, spectra of physical mixtures and co-ground products with amorphous Cds were very similar, both exhibiting no shifts but a very marked reduction in intensity of the typical bands of the drug. This was indicative of the high, homogeneous dispersion of the drug in the amorphous carrier which was obtained by simple blending, and scarcely improved by the co-grinding process.

Stability studies

Stability studies were performed in order to test the stability on ageing of the activated amorphous state of the drug obtained in co-ground systems with the different Cds and to reveal any possible transformation of the amorphous form to the crystalline one. Samples were stored at room temperature according the normal ageing conditions; in addition, the effect of high relative humidity (75% RH) was also evaluated, since this can be a possible critical factor for the stability of amorphous forms. The thermal profiles of co-ground products after storage in closed glass containers for four months at 25°C or after exposure for one month to the moisturized atmosphere were practically identical to those obtained for the corresponding freshly-prepared products (Fig. 5). In fact, the only evident difference was the increased intensity of the Cd dehydration band in samples stored at 75% RH. These results indicated that no crystalline rearrangements occurred in co-ground products during the storage period, not even in the presence of moisture, accounting for the suitability of the cyclodextrin co-grinding technique to obtain GLY in a stable activated amorphous form. On the other hand, DSC analyses performed on pure GLY samples, stored under the same experimental conditions for comparison purposes, showed that the crystallinity degree of the drug was unchanged, as demonstrated by practically unchanged temperature and enthalpy of its melting peak.

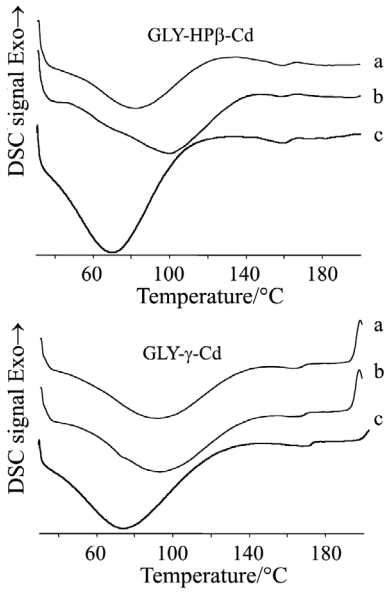

Fig. 5 DSC curves of some representative GLY-Cd 60 min co-ground (GR) mixtures: a – freshly-prepared; b – after storage in closed glass containers at 25°C for 4 months; c – after storage at 25°C and 75% RH for 1 month

Conclusions

Solid-state characterization of the different GLY–Cd systems demonstrated that co-grinding with Cds was able to give rise to an amorphous form of the drug, independent of the nature of the Cd used. Comparison of systems with natural crystalline or amorphous derivative Cds highlighted the higher drug amorphizing power of Cd-derivatives in the starting physical mixtures. However, this initial difference tended to diminish during the co-grinding process, since crystalline Cds showed themselves to be more susceptible to the mechanical treatment. In fact, all 60-min co-ground products were almost totally amorphous. Moreover, Cds seemed to act also as stabilizing agents of the more soluble amorphous form of the drug, by preventing, or at least slowing down, recrystallization phenomena during ageing, as was confirmed by the results of stability studies. No stability differences during storage were observed among samples with the different examined Cds. These results seem to indicate that the amorphous form of GLY, dispersed in the macrocyclic matrix carrier, is physically stable, in contrast to that obtained by spray-drying [10], thus making it possible for use in the development of solid dosage forms with improved drug dissolution properties.

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