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Characterization of glibenclamide glassy state

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Summary

Glibenclamide crystals were converted to the glassy state by cooling the melt on an ice bath. The glass state formation was confirmed by differential scanning calorimetry (DSC) measurements. Further evidence was provided by other techniques such as infrared spectroscopy (IR), X-ray powder diffraction, equilibrium solubility and dissolution studies. X-ray diffraction patterns of pulverized glassy glibenclamide stored up to 4 months at room temperature indicated no transformation to the crystalline state. The IR studies showed that the characteristic peaks of glibenclamide at 3310, 3100, 1725 and 1610 cm^{-1} decreased in intensity in the glassy state which was attributed to the partial transformation of the most stable keto form to the enol form during glass formation. Storing glassy glibenclamide at different temperatures resulted in a decrease in its equilibrium solubility. This decrease was attributed to the partial transformation of the glassy form to a crystalline form. The kinetics of the transformation process were pseudo first order in nature. Dissolution studies showed that glassy glibenclamide exhibits a higher dissolution rate than either crystalline glibenclamide or a commercially available product (Daonil).

Introduction

Glibenclamide (5-chloro-*N*-{2-[4-({[(cyclohexylamino)carbonyl]amino}sulfonyl)phenyl]ethyl}-2-methoxybenzamide) is a potent oral hypoglycemic agent and is frequently used in the treatment of diabetes mellitus (O'Sullivan and Cashman, 1970). Glibenclamide is virtually insoluble in water and exhibits very poor in vitro dissolution which has been thought to result in its low bioavailability (Chalk et al., 1986; Shaheen et al., 1987).

Several techniques have been employed to increase the solubility of poorly water soluble drugs. These include solid dispersions (Chiou and Regelman, 1971), the use of surface-active agents and hydrophilic polymers (Najib and Suleiman, 1985), molecular dispersions (Ganley et al., 1984), solid-state manipulations such as polymorphic transformation (Suleiman and Najib, 1989) and glass formation (Fukuoka et al., 1986, 1987).

The objective of this work was to investigate the glass forming capability for glibenclamide, characterization of the glassy material produced

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by cooling glibenclamide melt by infrared spectroscopy, X-ray powder diffraction, thermal analysis and thin layer chromatography. Equilibrium solubility and the rate of dissolution were also studied.

Materials and Methods

Materials

Glibenclamide was of pharmaceutical grade, was kindly provided by Dar Al-Dawa Development and Investment Company, Na'ur, Jordan. All reagents used were of analytical grade. Double-distilled water from an all glass still was used in the equilibrium and dissolution studies.

Methods

Preparation of glass

Glibenclamide crystals were melted in a beaker by heating on a paraffin oil bath maintained at 185°C and the melt was solidified by cooling with stirring on an ice bath. The solidified melt formed was placed in a desiccator over silica until use.

Thin-layer chromatography (TLC)

The chemical stability of glibenclamide during preparation of the solidified melt was studied using TLC. Glibenclamide samples were dissolved in methanol and spotted on silica gel 60 F254 plates (E. Merck, Darmstadt, F.R.G.), which were developed with two solvent systems; (A) benzene : glacial acetic acid : ethyl acetate : acetone (65 : 6 : 12 : 30), (B) chloroform : cyclohexane : ethanol : glacial acetic acid (9 : 9 : 1 : 1) and were detected by placing the plates in a chamber containing iodine vapor or under ultraviolet (UV) light.

Infrared spectroscopy (IR)

Potassium bromide disks of the samples (2%) were prepared using a Shimadzu hand press and spectra were recorded on an IR-435 Shimadzu spectrophotometer (Shimadzu, Kyoto, Japan).

X-ray powder diffraction

The X-ray powder diffraction patterns were recorded on a Philips PW 1050/81 Goniometer with a PW 1729 Generator, a PW 1710 Diffractometer controller and a PM 8203 A recorder under the following conditions: Ni filtered Cu-K_α radiation ($\lambda = 1.54186$ Å); voltage, 30 kV; current, 40 mA; slit width, 0.5°; scale, 5000.

Differential scanning calorimetry (DSC)

The DSC curves were recorded on a TA 3000 Mettler thermal analyzer, equipped with a DSC cell, under static conditions. Samples (5 ± 1 mg) were placed in open aluminum crucibles and heated linearly at 10°C min^{-1} from ambient temperature to 200°C against an empty crucible as the reference.

Equilibrium solubility studies

Samples of 5 mg glibenclamide (0.125–0.2 mm sieve) were placed in 10 ml phosphate buffer (pH 7.4) in glass-stoppered Erlenmeyer flasks. The flasks were agitated at 100 strokes/min in a thermostated shaking water bath (Karl Kolb, Dreieich, F.R.G.) adjusted at 37 ± 0.5°C. After 2 days the samples were filtered through a 0.45 μm Millipore filter (Whatman Ltd, Maidstone, U.K.). The concentration of glibenclamide in each sample was determined photofluorometrically using Nova Spectrofluorimeter (Baird Atomic Ltd, Essex, U.K.) when excited by radiation of wavelength 308 nm and measuring the emitted fluorescence at 360 nm with reference to a suitably constructed standard curve.

Dissolution studies

The dissolution rate studies were performed in a USP XX type 2 dissolution apparatus (DT-D6, Erweka, F.R.G.) in 500 ml phosphate buffer solution (pH 7.4) maintained at 37 ± 0.5°C. The stirring rate was 100 rpm. Each sample powder (0.125–0.2 mm sieve) of glibenclamide was placed in the dissolution medium. At appropriate intervals 5 ml aliquots were withdrawn, filtered rapidly through a Millipore filter (0.45 μm) and assayed photofluorometrically as outlined above. A correction was applied for the cumulative dilution caused by replacement of the sample by equal volumes of

the original medium. The experiment was carried out in triplicate.

Results and Discussion

The obtained solidified melt of glibenclamide was a transparent and brittle glassy mass. To examine the effect of melting on the chemical stability of glibenclamide, the crystalline and solidified melt samples were studied by TLC method using two solvent systems. The R_f values were 0.73 and 0.53 for the solvent systems A and B, respectively. Both the crystalline and the glassy samples gave a single spot with the same retention time, indicating that decomposition of the drug did not occur during the melting process.

The DSC thermal curves of the crystalline materials and the glassy state are presented in Fig. 1. The DSC thermogram (Fig. 1a) of crystalline glibenclamide shows one endothermic peak at 174.4°C corresponding to its melting point. The DSC thermogram of the solidified melt (Fig. 1b) shows one shallow and broad peak. This suggests that a glass was formed under the employed conditions. The glass transition temperature of glibenclamide (T_g) as determined from DSC measurements was 71.3°C (an average of four readings). The purity of the glassy form was determined by DSC to be 99.4 ± 0.3% (mean ± S.D, $n = 5$). This was supported by TLC and IR studies.

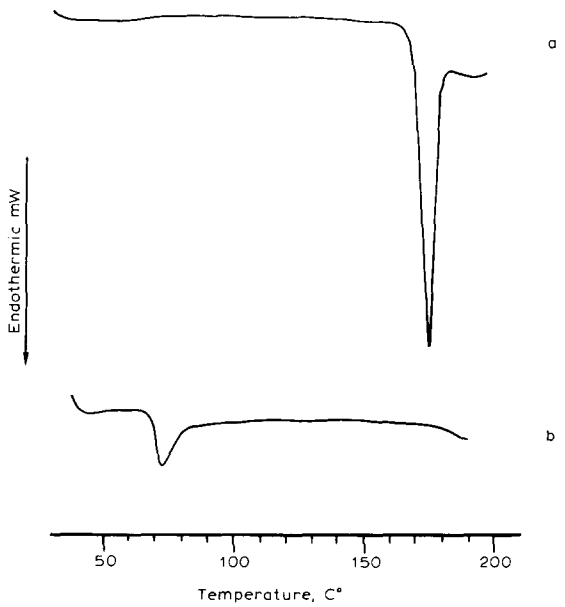


Fig. 1. DSC curves of glibenclamide: a, crystalline; b, glass.

The X-ray diffraction pattern of the crystalline glibenclamide (Fig. 2) shows a strong and sharp peaks. Meanwhile, the diffraction pattern of the glassy state produced weak and diffuse diffraction spectra. These results further confirm that the solidified melt was a glass and of an amorphous nature.

In order to study the effect of grinding on the transition of glibenclamide from glass to crystalline form, glibenclamide glass was pulverized in a mortar and crystals (0.125–0.2 mm sieve) were collected and stored at room temperature in a desiccator over silica until use. The X-ray diffraction patterns of pulverized glassy glibenclamide stored up to four months did not show any increase in the intensity of peaks. This indicated that the glassy state did not undergo any crystallization under the conditions used.

The IR spectrum of crystalline glibenclamide (Fig. 3a) is similar to the previously published spectra (Takla, 1981). The absorption peaks characteristic of urea, sulphonamide, and amide N-H stretching were observed at 3360, 3100 and 3310 cm^{-1}, respectively. The carbonyl groups of urea and amides show absorption peaks at 1610 and 1725 cm^{-1}, respectively. The absorption peaks at 1515 cm^{-1} are due to urea N-H bending and the amide II band. The sulphonyl SO_2 group shows strong absorption bands at 1330 cm^{-1} for the asymmetric stretching and at 1160 cm^{-1} for the symmetric stretching. The ether C-O-C group shows strong absorption bands at 1245 cm^{-1} and 1020 cm^{-1} for the asymmetric and symmetric stretching vibrations, respectively. The IR spectrum of the glassy material (Fig. 3b) exhibits significant differences in the intensities of certain absorption peaks. A slight change in the position of most absorption peaks and broadening of peaks was also observed; particularly a decrease in the intensity of absorption peaks characteristic of the sulphonamide N-H, amide N-H, urea carbonyl and amide carbonyl groups. This large decrease in

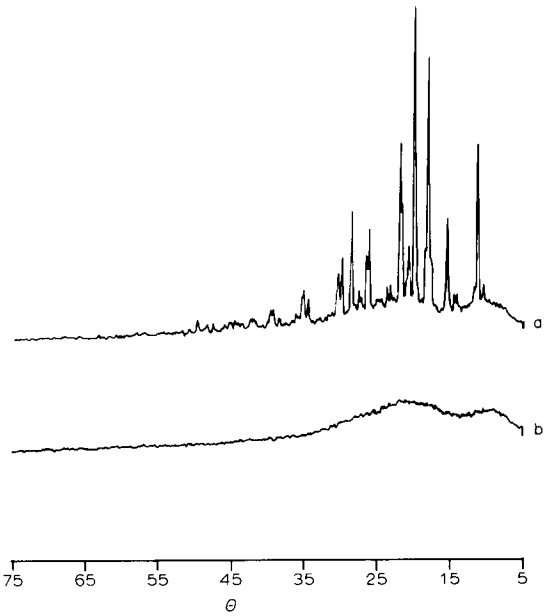


Fig. 2. X-ray diffraction patterns of glibenclamide: a, crystalline; b, glass.

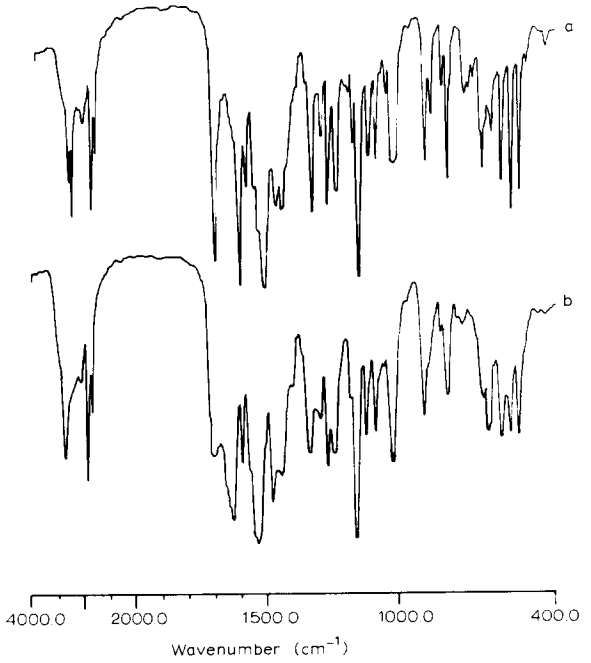
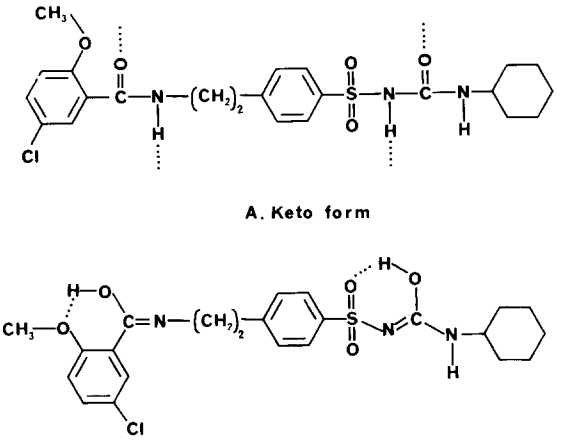


Fig. 3. IR spectra of glibenclamide: a, crystalline; b, glass.

intensity of these peaks could be due to partial transformation of the most stable *keto* form in the commercial glibenclamide (A), which is stabilized



Scheme 1. *Keto-enol* tautomerism.

by NH · · · O intermolecular hydrogen bonding, to the thermodynamically less stable *enol* form (B) during glass formation, which could be stabilized by intramolecular hydrogen bonding between the O-H and the SO_2 groups and/or between the O-H group and the oxygen of the O-methoxy substituent, to form a six-membered ring structure as suggested in Scheme 1. A similar behavior was observed in acetohexamide polymorphs, a sulphonyl urea, which was also reported to exhibit *keto-enol* tautomerism (Takla, and Dakas, 1989). The partial transformation of the *keto* to the *enol* form would result in the partial loss of the sulphonamide N-H, the amide N-H, the amide carbonyl and urea carbonyl absorption peaks, and the appearance of absorption peaks for the intramolecularly hydrogen bonded O-H groups, together with a change in the intensities and positions of the sulphonyl SO_2 and ether C-O-C absorption peaks. The intramolecularly hydrogen bonded absorption peaks of the hydroxyl group in the *enol* form, which is usually broad and shallow, was not observed in the IR spectrum of the glass form. This could be explained by the possible overlap with urea N-H absorption peak appearing at 3360 cm^{-1}. A decrease in the intensities of sulphonamide SO_2 and ether C-O-C absorption peaks with a slight shift in the position to a lower wave length was observed which is attributed to the intramolecular hydrogen bonding.

The conformation of the commercially available crystalline glibenclamide was reported to be dominated by NH · · · O hydrogen bonds (Byrn et al., 1986). The hydrogen bonding is primarily responsible for glass formation by preventing recrystallization (Fox et al., 1963). The possibility for more than one kind of intermolecular hydrogen bonding in glibenclamide together with the possibility of *keto-enol* tautomerism and intramolecular hydrogen bonding would explain the glass forming capability of glibenclamide. Hence, the glassy state may be composed of a mixture of conformations responsible for broadening and splitting of peaks, as it was actually observed in the IR spectrum of the glassy state.

The effect of aging on the IR spectrum of glibenclamide glass is shown in Fig. 4. Samples of glibenclamide glass powder (0.125-0.2 mm sieve) were stored at 50°C (a), 60°C (b) and 70°C (c) for a period of 2 months. Fig. 4 indicates that a change in the intensities of certain absorption peaks has occurred. The peaks which exhibited decreased intensity as a consequence of glass state transformation, particularly those at 3310, 3100, 1725 and 1610 cm^{-1}, increase in intensity with time and with the increase in storage temperature, which indicates the partial retransformation of the *enol* form to the *keto* form.

The IR spectrum of glibenclamide crystals obtained by recrystallization of the glassy form from acetonitrile was found to be identical to the IR spectrum of the commercial sample, which indicates the complete transformation of the glass to the *keto* form. This provides additional support to the chemical stability of glibenclamide during the melting process.

The effect of aging on the DSC thermal curve of glibenclamide glass was also studied (Fig. 5). This figure indicates the partial transformation of the amorphous form to the crystalline form on storage at 50°C (a), 60°C (b) and 70°C (c) for 2 months. The extent of transformation increases with the increase in storage temperature. This confirms the results obtained from the IR studies on the glassy form.

The equilibrium solubility of crystalline glibenclamide in phosphate buffer (pH 7.4) at 37°C was 0.01 mg/ml. The glassy form shows a solubility of

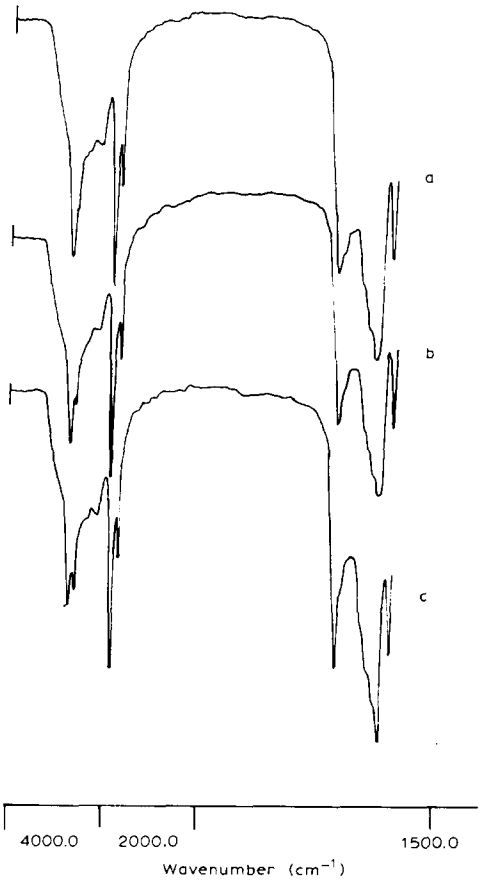


Fig. 4. IR spectra of glibenclamide glass stored at different temperatures for 2 months: a, 50°C; b, 60°C; c, 70°C.

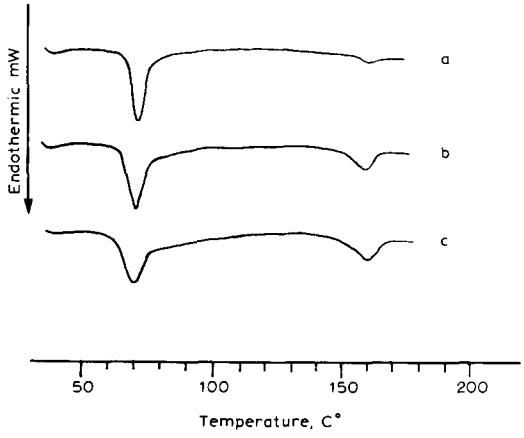


Fig. 5. DSC curves of glibenclamide glass stored at different temperatures for 2 months: a, 50°C; b, 60°C; c, 70°C.

0.1 mg/ml, indicating a 10-fold increase in solubility.

Fig. 6 shows the equilibrium solubility of glibenclamide glass stored at different temperatures for different durations. A curvilinear relationship was obtained which indicates that for any one storage temperature the solubility initially decreases as the storage time increases up to 21 days. However, as the storage temperature increases from 40 to 70 °C the extent of decrease in solubility increases. Increasing the storage time for more than 21 days results in very little reduction in solubility.

The reduction in solubility of glibenclamide glass when stored at different temperatures was attributed to the partial transformation of glibenclamide from the amorphous glassy state to the crystalline state. As the amorphous state is more energetic and less stable than the crystalline state as indicated from the heat of fusion ΔH_f values a decrease in solubility was noted. The ΔH_f values as calculated from DSC data were 18.7 and 94.0 J g^{-1} for the amorphous and crystalline states respectively. Storage of crystalline glibenclamide at the same temperatures and for the same periods of time as the glassy form did not induce any significant changes as indicated from the equilibrium solubilities and confirmed by DSC and IR studies.

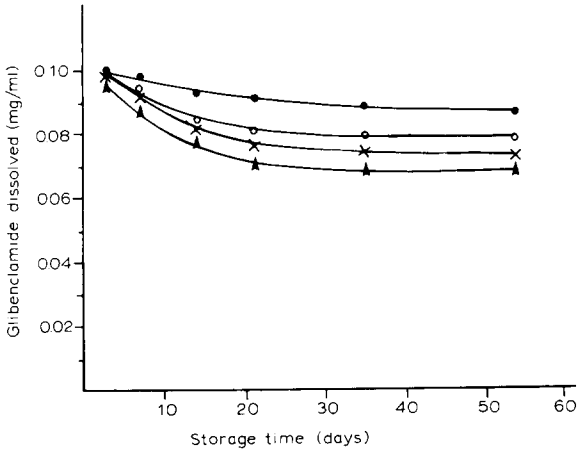


Fig. 6. Equilibrium solubility of glibenclamide glass stored at different temperatures as a function of time [(●) 40°C; (○) 50°C; (×) 60°C; (▲) 70 °C].

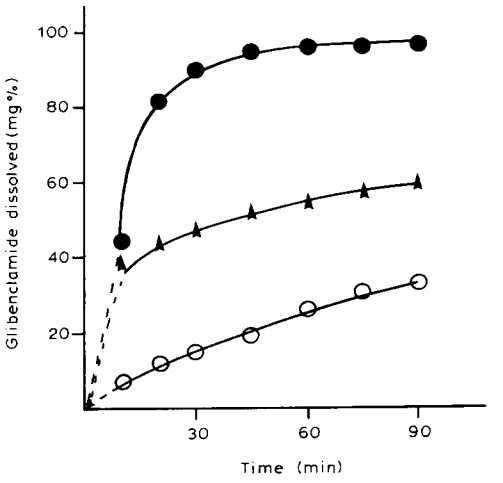


Fig. 7. Dissolution profiles of: (●) crystalline glibenclamide; (▲) Daonil; (○) glibenclamide glass.

The dissolution profiles of glibenclamide glass, crystalline and a commercially available glibenclamide product (Daonil, Hoechst), are shown in Fig. 7. It is evident that the glibenclamide glass demonstrates a faster dissolution rate than the crystalline form or Daonil. At 30 min, the amount dissolved from the glass was 90% compared to 15 and 47% from crystalline glibenclamide and Daonil, respectively. Complete dissolution of glibenclamide glass was observed in less than 45 min. Meanwhile, after 90 min the dissolution of crystalline glibenclamide and Daonil was less than 35 and 60%, respectively. These results indicate a significant enhancement in the dissolution rate of glibenclamide by the use of its glassy state, which would possibly result in an improvement of its bioavailability.

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