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Inclusion complexation of glisentide with α-, β- and γ-cyclodextrins

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Abstract

Complexation of glisentide with α-, β- and γ-cyclodextrin (CD) has been investigated in aqueous solution and in the solid state. Complex formation in solution has been analysed using solubility diagrams and NMR spectroscopy and the interaction in solid state has been studied by X-ray diffractometry, DSC and IR spectroscopy. The thermodynamic parameters, $\Delta H°$, $\Delta S°$ and $\Delta G°$, of complexation with β- and γ-CD have been calculated from the temperature dependence of the stability constant. The process has been found to be exothermic and $\Delta S°$ is slightly unfavourable. In addition, it has been found that the ionization state of glisentide plays an important role in complexation and the fact that the extent of complexation is greater with β- than with γ-CD has revealed the importance of the cavity size to get an adequate fitting between host and guest molecules. The inclusion of the *ortho*-substituted aromatic ring of glisentide has been evidenced by NMR spectroscopy. Finally, complexes have been prepared by coprecipitation and kneading methods and it has been found that the former is more suitable to achieve solid-state complexation.

Keywords: Glisentide; Cyclodextrins; Inclusion complexation; Complex stability constant

Glisentide is a second-generation sulfonylurea that is indicated for the treatment of non-insulin-dependent diabetes mellitus. Its chemical structure is closely related to glibenclamide. The drug is poorly soluble in water and its dissolution is considered to be a rate-determining step in its absorption from the gastrointestinal fluids.

The aim of this study was to characterise the interactions between glisentide and α-, β- and γ-cyclodextrins (CD). The extent of complexation in solution has been evaluated by determining the apparent stability constants of the respective complexes, and the structure of the complex with β-CD has been elucidated using NMR spectroscopy. Finally, complexes with α-, β- and γ-CD have been characterised in the solid state.

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Glisentide was kindly supplied by Uriach-Biohorm (Barcelona, Spain), α- and γ-CD were purchased from Wacker Chemie (Barcelona, Spain) and β-CD from Roquette-Freres (Barcelona, Spain). All the reagents were of analytical grade.

As the drug is a weak acid ($pK_a = 6.3$), the phase solubility studies were carried out in pH 4.2 and 8.6 phosphate buffers (Na_2HPO_4, KH_2PO_4). Twenty-five mg of glisentide were weighed out in 25-ml glass tubes containing different concentrations of cyclodextrin. The tubes were placed in a bath at constant temperature and shaken until equilibrium was reached (24 h); then the solutions were filtered (0.8 μm pore) and the concentration of glisentide was spectrophotometrically determined at 230 nm with pH 4.2 solutions and at 226 nm with pH 8.6 solutions, using an HP 8452 A diode array spectrophotometer. The presence of the ligands did not interfere in the absorption measurements.

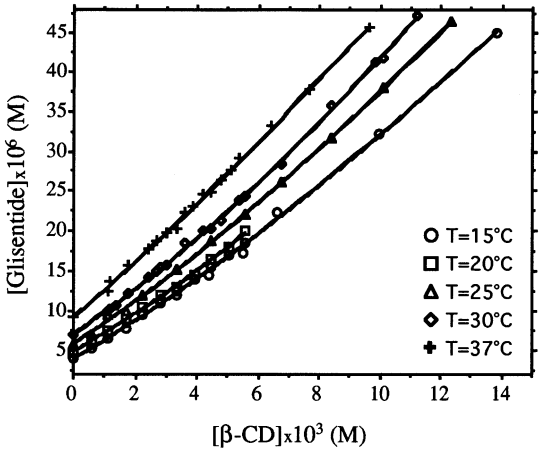

Fig. 1. Solubility diagrams of glisentide–β-CD complex system in pH 4.2 aqueous solution at different temperatures.

Table 1
Apparent stability constants ($K_{1:1}$) for the complexation of glisentide with β- and γ-CD in pH 4.2 aqueous solution at different temperatures

T (°C)	$K_{1:1}$ (M^{-1})	
	β-CD	γ-CD
15	660 ± 30	145 ± 8
20	570 ± 40	
25	510 ± 20	98 ± 5
30	460 ± 20	86 ± 4
37	390 ± 10	66 ± 3

Values are the mean ± S.E. of four determinations.

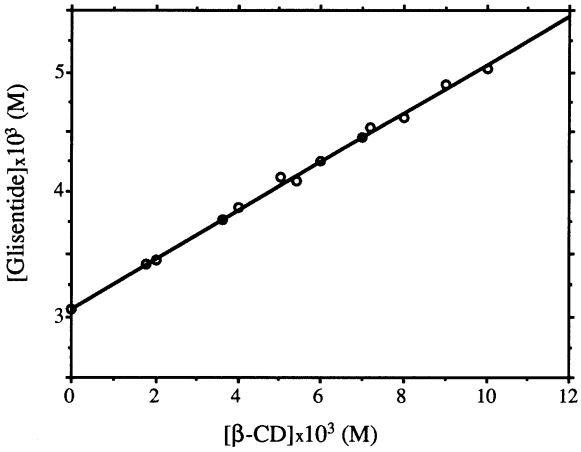

Fig. 2. Solubility diagram of glisentide–β-CD complex system in pH 8.6 aqueous solution at 25°C.

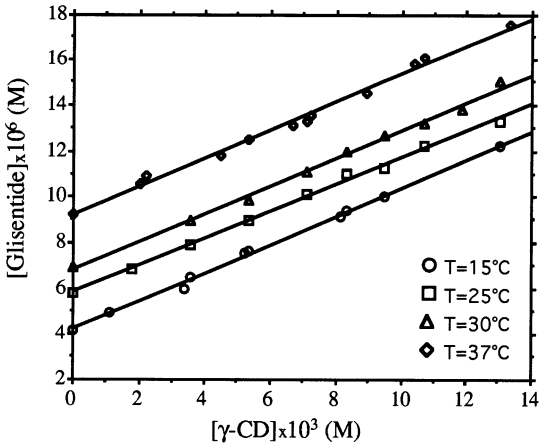

Fig. 3. Solubility diagrams of glisentide–γ-CD complex system in pH 4.2 aqueous solution at different temperatures.

The phase solubility diagrams obtained for the complex with β-CD in an acid medium can be classified as A_P type (Fig. 1). The apparent stability constants of the complex (Table 1) were calculated from the initial straight line portion of the diagram, assuming a 1:1 stoichiometry (Higuchi and Connors, 1965).

In pH 8.6 buffer, glisentide is ionized and its phase solubility diagram (Fig. 2) can be classified as A_L type. The apparent stability constant at

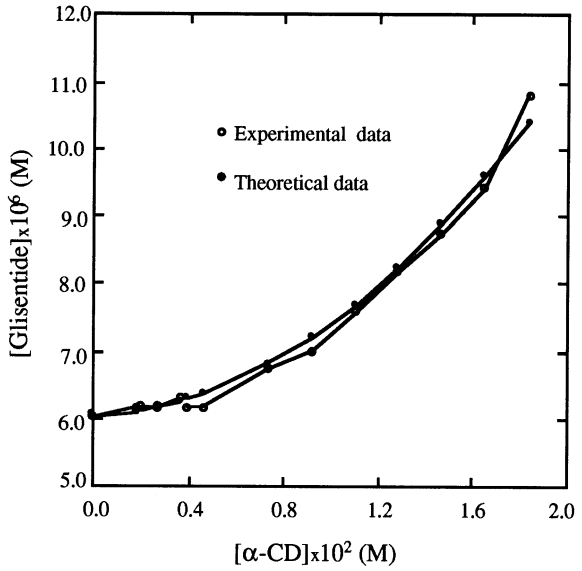

Fig. 4. Solubility diagram of glisentide–α-CD complex system in pH 4.2 aqueous solution at 25°C.

25°C was $K_{1:1} = 80 \pm 4\ M^{-1}$ ($r = 0.998$) while at pH 4.2 it was $K_{1:1} = 510 \pm 20\ M^{-1}$. Therefore, it can be concluded that the ionization state of glisentide plays an important role in complexation, probably due to the fact that the ionized form of the drug is less capable of displacing the water molecules of the cavity to yield more stable systems. The results obtained with β-CD are in agreement with the stability constant in water ($K_{1:1} = 200\ M^{-1}$) reported by Moyano et al. (1995).

Complexation with γ-CD in pH 4.2 solution gives rise to A_L type solubility diagrams (Fig. 3) at all the temperatures studied, and a 1:1 stoichiometry can be assumed for determining the apparent stability constants which are shown in Table 1.

Complexation with α-CD has been studied in pH 4.2 aqueous solution at 25°C. The A_P type solubility diagram (Fig. 4) suggests the formation of a 1:2 glisentide–α-CD inclusion complex. The possible formation of this complex involves a small enhancement in the solubility of glisentide which involves a considerable experimental error (variation coefficient of the solubility measurements 8%) so the apparent stability constants could not be accurately determined.

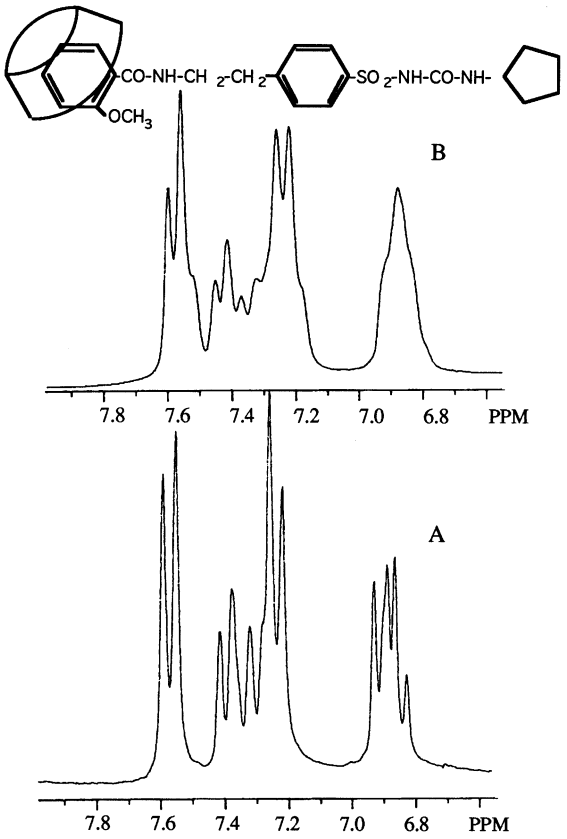

Fig. 5. NMR spectra (200 MHz) of glisentide (A) and the inclusion complex with β-CD (B). Proposed structure for the complex.

Table 2
Thermodynamic parameters of complexation with β- and γ-CD in pH 4.2 aqueous solution

Complex	$\Delta H°$ (kJ mol^{-1})	$\Delta S°$ (J mol^{-1} K^{-1})	$\Delta G°$ (kJ mol^{-1})	r
Glisentide–β-CD	-17.4 ± 0.5	-6.6 ± 1.6	-15.4 ± 0.9	0.999
Glisentide–γ-CD	-26.0 ± 0.9	-50 ± 4	-11.1 ± 2	0.998

Standard errors are given.

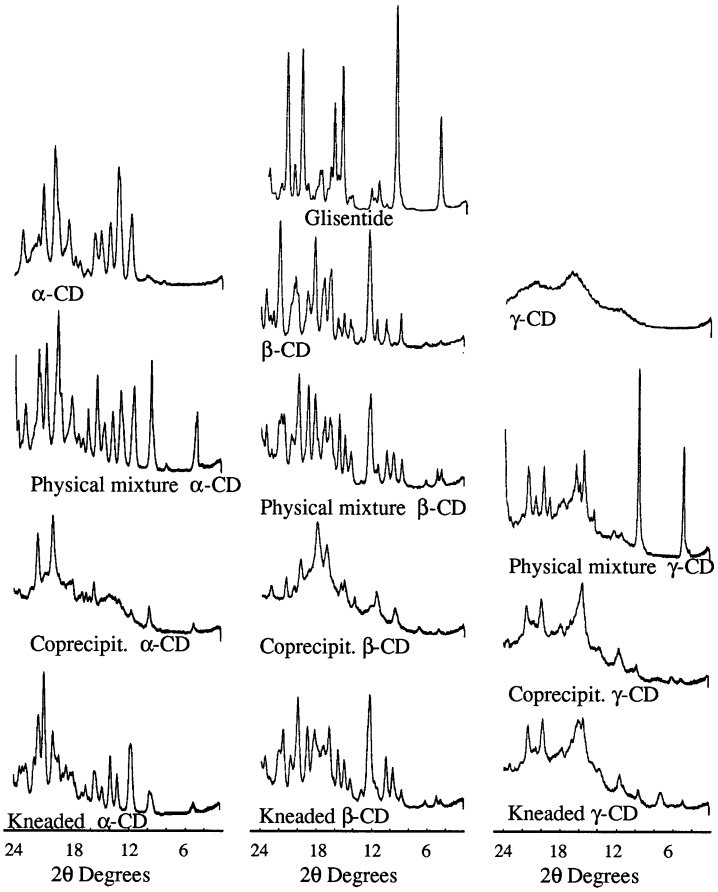

Fig. 6. X-ray diffraction patterns of glisentide, α-, β- and γ-CD and their respective physical mixtures, coprecipitates and kneaded systems.

The differences obtained in the complexation of glisentide with α-, β- and γ-CD reveal the importance of the cavity size to get an adequate fitting between host and guest molecules.

The apparent values of enthalpy ($\Delta H°$) and entropy ($\Delta S°$) changes of complexation with β- and γ-CD were calculated from linear plots of $\ln K_{1:1}$ versus $1/T$, according to the integrated form of the Van't Hoff equation (Gelb et al., 1981). Complexation involves favourable enthalpy changes and unfavourable entropy changes (Table 2). The high enthalpy values may suggest that hydrogen bonds between host and guest participate in the formation of the complexes and the negative entropy changes can be associated to an increase of order in the system when complexation takes place.

The structure of the complex with β-CD has been elucidated using NMR spectroscopy (Bruker AC 200 spectrometer). The solvent used was deuterium oxide (Merck, Barcelona, Spain) containing the minimum amount of sodium hydroxide-d1 (Merck) to achieve the solubilization of glisentide. The internal reference was the peak due to small quantities of DHO and H_2O which was assigned to $\delta = 4.8$ ppm.

Fig. 5 shows that signals of protons belonging to the terminal aromatic ring of glisentide (7.30–7.45 ppm) experience a downfield shift in the complex. This shift can be attributed to variations of the local polarity when these protons are inside the cavity. Taking into account the steric hindrance and polarity of the methoxy group, a structure can be proposed for the complex (Fig.

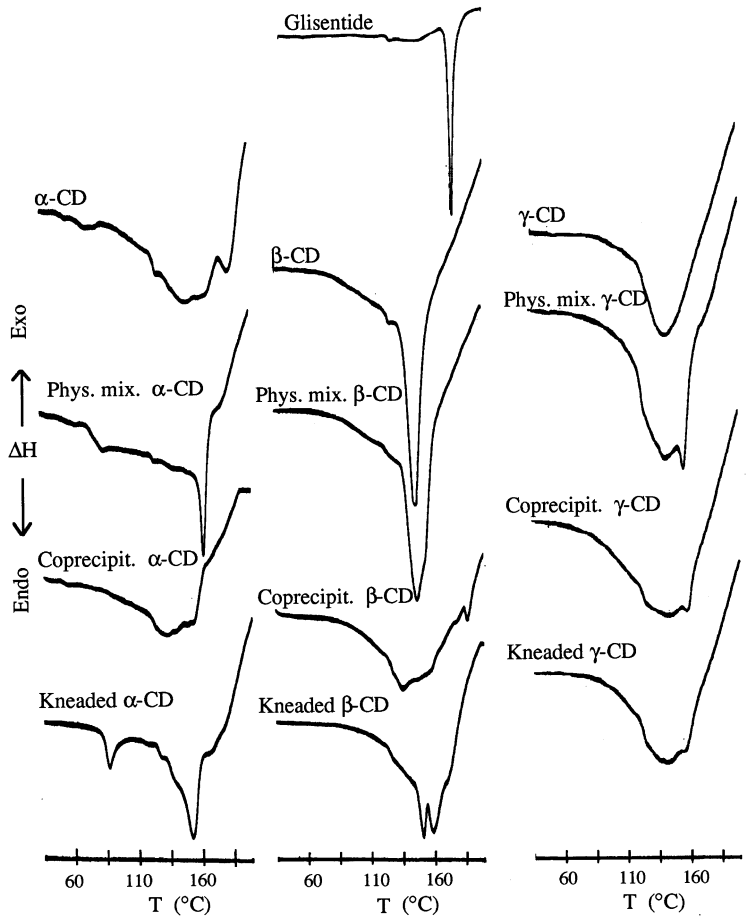

Fig. 7. DSC thermograms of glisentide, α-, β- and γ-CD and their respective physical mixtures, coprecipitates and kneaded systems.

5). These NMR spectral modifications are in agreement with the results obtained in a study of the complex glibenclamide–β-CD carried out by Redenti et al. (1995), who also reported the inclusion of the aromatic ring. It is well known that β-CD offers the optimum cavity environment for binding phenyl rings (Gadre et al., 1997).

Finally, solid complexes of glisentide with α-, β- and γ-CD in 1:1 molar ratios were prepared by coprecipitation and kneading:

(1) Coprecipitation: 1:1 stoichiometric quantities of glisentide and cyclodextrin were dissolved in water containing the minimum amount of 1% NH_4OH aqueous solution to achieve the dissolution of the drug, the pH was 10 so glisentide was in salt form. The solvent was eliminated by vacuum evaporation at 70°C.

(2) Kneading method: 1:1 stoichiometric quantities of glisentide and cyclodextrin were thoroughly mixed in a mortar and an amount of water equal to the weight of the drug–ligand mixture was added. It was stirred during 1 h in order to get a paste which was subsequently dried at 50°C during 24 h.

The characterization of solid complexes of glisentide with α-, β- and γ-CD was carried out by powder X-ray diffraction analysis (Siemens Kristalloflex 810 diffractometer, Cu Kα radiation; voltage 40 kV; current 20 mA; time constant 4 s; scanning speed 1° cm^{-1}), DSC (Setaram DSC-92, with a scanning rate of 5°C min^{-1}) and IR spectroscopy (Perkin-Elmer 681 spectrometer in KBr pellets).

The possible formation of solid complexes has

been studied by comparison with the corresponding physical mixtures. The X-ray diffraction patterns (Fig. 6) show that the coprecipitated systems are almost amorphous, whereas the kneaded products present a diffraction pattern which is quite similar to that of the physical mixture, although some peaks have disappeared, specially in the system with γ-CD. In general, complexation with other sulfonylureas has been reported to involve a decrease in the degree of crystallinity (Vila-Jato et al., 1987; Gandhi and Karara, 1988; Torres-Labandeira et al., 1993; Sanghavi et al., 1994).

The DSC curves of glisentide with α-, β- and γ-CD are shown in Fig. 7. The endothermic peak corresponding to the melting of glisentide, which is present at 160°C approximately in the physical mixtures, disappeared in the coprecipitated products, this fact evidences the formation of complexes in the solid state. This behaviour is not clearly observed in the kneaded preparations as the melting peak of glisentide is only slightly reduced.

The IR spectra of the systems with α-, β- and γ-CD showed slight modifications upon complexation in the aromatic region between 1235 and 1500 cm^{-1} and in the broadening of the band at 1715 cm^{-1}, which corresponds to the aromatic carbonyl stretching region so this analytical technique is not as useful as the above mentioned to evidence the complexation of glisentide.

It can be concluded that coprecipitation is more appropriate than kneading to achieve complexation.

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