Glibornuride-β-Cyclodextrin Inclusion Complexes: Preparation, Structural Characterization and in vitro Dissolution Behaviour

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Summary

Phase-solubility studies of the complexation of glibornuride (GB) with α-, β- and γ-cyclodextrin (CyD) showed that the most stable complex is formed with β-CyD. The effects of β-CyD on chemical shift values in ^{1}H-NMR spectra of GB in aqueous medium suggest that it is the bornyl part of the drug which is bound inside the CyD cavity. Solid inclusion complexes were obtained by coprecipitation and freeze-drying methods, and characterized by X-ray diffractometry and differential scanning calorimetry. Drug solubility was improved by inclusion in solid complexes obtained by either method.

1 Introduction

Cyclodextrins (CyDs) are cyclic oligosaccharides formed from six or more units of D-glucose linked by α-(1-4) glucosidic bonds. The most commonly used CyDs contain 6, 7 or 8 glucose units (α-, β- and γ-cyclodextrin, respectively). CyDs are doughnut-shaped with a central cavity that can accommodate one or several molecules, which are held by non-covalent interactions (1–3). Inclusion of drug molecules in the CyD cavity has received considerable attention as a means of improving the solubility, chemical stability and bioavailability of the included drugs (4–8).

The structure of inclusion complexes of sulphonylureas with β-CyD has been investigated by techniques including circular dichroism, Raman, ^{1}H- and ^{13}C-NMR spectroscopy (9–13). These inclusion complexes with β-CyD have been shown to increase the bioavailability of the drug (13–15).

Glibornuride (GB) (endo, endo-1-(2-hydroxy-3-bornyl)-3-(p-tolylsulphonyl) urea) (Fig. 1) is a sulphonylurea with very low hydrosolubility (< 12 μg ml^{-1}); its inclusion in β-CyD increases bioavailability following oral administration to rabbits (15). The phenylic part of the drug is bound within the β-CyD cavity by a hydrophobic interaction (10, 11). However, borneol has also been shown to form inclusion complexes with β-CyD, and this im-

proves its stability (16). One possibility is that GB is simultaneously included in the cavities of two β-CyD molecules, leading to a 1:2 (GB:β-CyD) stoicheiometry.

In this study we used phase-solubility techniques to analyse the complexation of the three natural CyDs (α-, β- and γ-CyD) with GB. Coprecipitation and freeze-drying methods were employed to obtain solid complexes of GB with β-CyD (which showed the highest capacity for complexation). ^{1}H-NMR, dissolution studies, X-ray diffractometry and differential scanning calorimetry (DSC) were then used to investigate the interaction between GB and β-CyD in aqueous solution and in the solid state.

2 Materials and Methods

2.1 Materials

Glibornuride was kindly supplied by Roche S.A., (E-Barcelona) and α-, β- and γ-cyclodextrins were purchased from Chinoin (H-Budapest). All other reagents were of analytical grade.

2.2 Phase solubility studies

The method described by Higuchi and Connors (18) was used. Different concentrations of α-,β-, and γ-CyD in 5 ml of simulated gastric fluid without pepsin (SGF) (pH 1.2 ± 0.1) (17) were added to an excess of GB (15 mg) in 5 ml ampoules. The trials were carried out in an acid medium to ensure that the drug — which is weakly acidic in solution ($pK_a = 5.3$) — was not ionized, and to promote complex formation (20). Three replicate trials were carried out for each concentration. The ampoules were mechanically shaked in a water bath at 25 ± 0.5 °C for 48 h to reach equilibrium. The solutions were filtered (0.45 μm) and the concentration of GB was determined by U.V. spectroscopy using a Shimadzu U.V.-240-Graphicord spectrophotometer (Shimazu Corp., J-Kioto) at 228 nm (ε = 13604).

The stability constant, K_s, was calculated from the slope of the initial linear section of the solubility diagram (16) as:
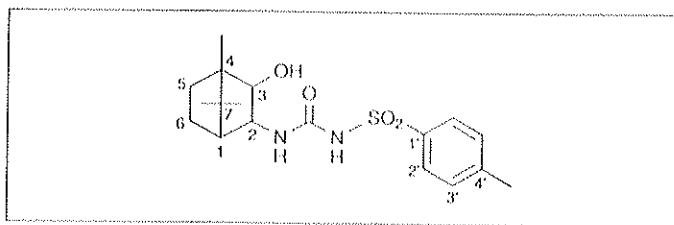
$$K_s = slope/[S_0(1\text{-}slope)]$$

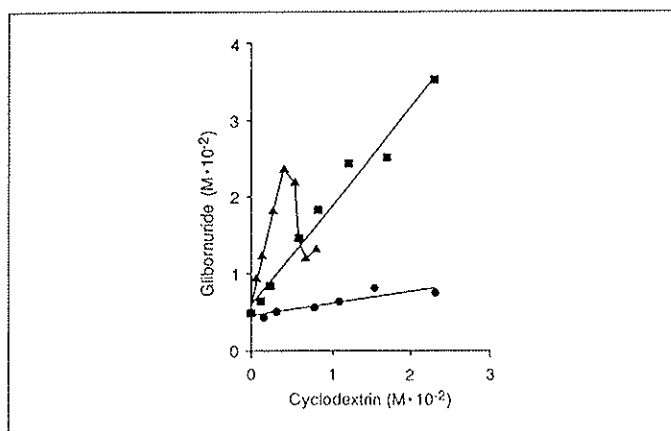**Fig. 1 Chemical structure of glibornuride**

Fig. 2 Phase solubility diagrams of glibornuride and one of the three natural cyclodextrins
● α-CyD; ▲ β-CyD; ■ γ-CyD

where S_0 is the solubility of the pure drug and assuming 1 : 1 stoicheiometry.

2.3 Preparation of solid complexes (1 : 1)

Coprecipitation method. GB (8.81 × 10^{-3}M) was added to 50 ml of SGF. β-CyD was then added and the mixture was mechanically shaked for 7 d to reach equilibrium. The solution was filtered and the filtrate dried under vacuum at 50 °C for 24 h. The stoicheiometry of the compound obtained was determined spectrophotometrically.

Freeze-drying method. GB (8.81 × 10^{-3}M) and β-CyD in a 1 : 1 molar ratio were dissolved in 0.14% aqueous ammonium hydroxide. The solution was filtered (0.45 μm) and frozen by immersion in liquid nitrogen. Freeze-drying was completed in 24 h in a Lyph-lock 6 apparatus (Labconco, USA-Kansas, MO). No trace of ammonium ions was detected in the compound obtained (nesslerization method).

2.4 ¹H-NMR studies

Nuclear magnetic resonance spectra were obtained with a Bruker WN 250 spectrometer (Bruker Anal. Messtechnik GmbH, D-Rheinstetten) at 25 °C. Samples were dissolved in 0.2 N deuterated sodium hydroxide. The internal reference was the peak due to small amounts of DHO and H_2O present as impurities, assigned a value of $\delta = 4.8$ ppm.

2.5 Characterization of the inclusion complex in the solid state

Thermal analysis. DSC was performed with a DSC-4 apparatus (Perkin Elmer, USA-Norwalk, CO) at a scanning rate of 10 °C · min^{-1}.

X-ray diffraction patterns. X-ray powder diffraction patterns were obtained using a Siemens d-500 diffractometer (Siemens, D-Karlsruhe) with Cu-K_a radiation monochromatized with a nickel filter.

2.6 Dissolution study

The dissolution rates of GB, of a 1 : 1 physical mixture of GB and β-CyD and of the GB/β-CyD complexes obtained as described above were determined as per Nogami et al. (19). A powdered sample containing 10 mg of drug (pure, physical mixture or its complexed form) was placed in 50 ml of SGF in a cell at 37 °C with shaking at 350 r.p.m. At predetermined times
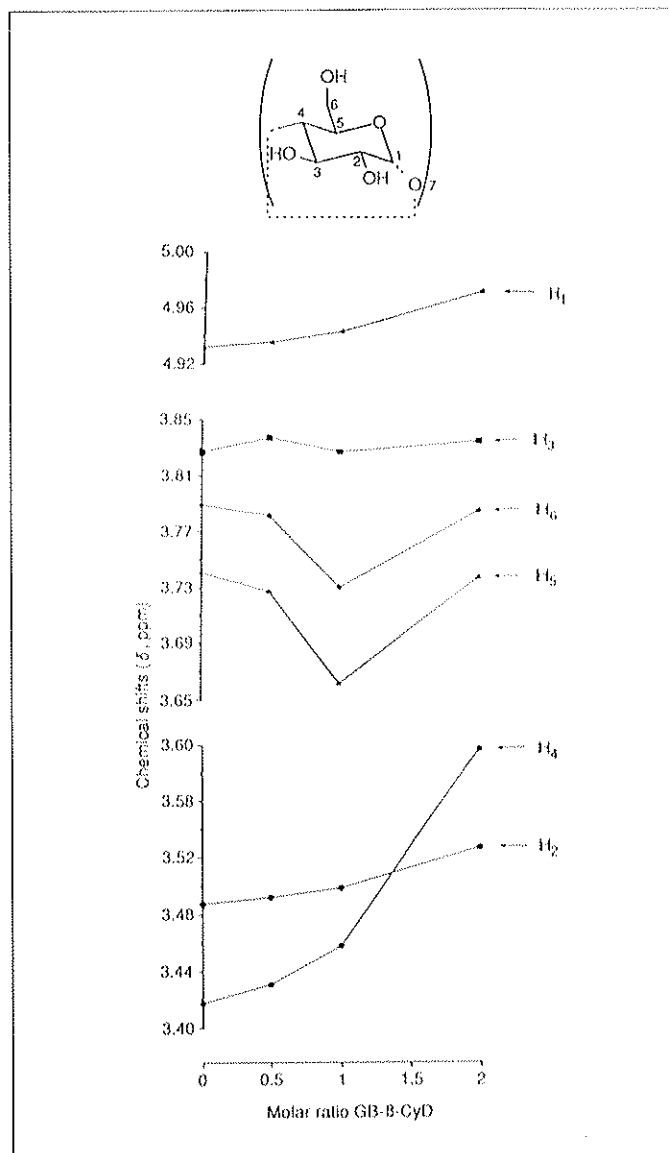

Fig. 3 Variation of ¹H chemical shifts of β-CyD in solutions varying in glibornuride concentration

samples were taken, filtered and diluted, and the concentration of GB determined spectrophotometrically. The experiments were carried out in triplicate.

Dissolution curves were characterized by dissolution efficiency (20), and results were evaluated by analysis of variance (ANOVA), and the least significant difference (LSD) test.

3 Results and Discussion

Solubility study. Fig. 2 shows the solubility phase diagrams for GB with the three CyDs. For α-CyD, solubility increases slowly and linearly with cyclodextrin concentration, and the curve may be classed as type A_L (18). The curve for γ-CyD is qualitatively similar, though the magnitude of the solubility increase is greater. The curve for β-CyD was of type B_S, the descending portion being accompanied by precipitation of the microcrystalline complex.

K_s values were 29.87 M^{-1} for α-CyD, 1737.24 M^{-1} for β-CyD and 268.30 M^{-1} for γ-CyD. The complex between GB and β-CyD had the highest stability and was thus selected for use in all subsequent experiments.
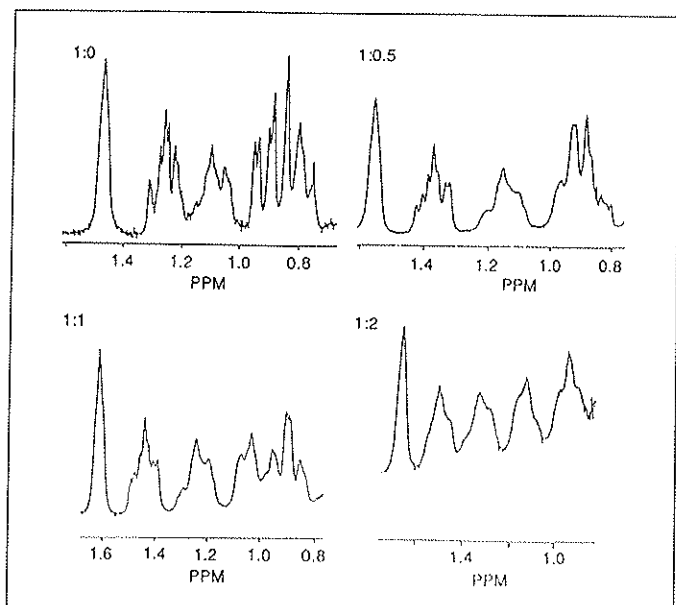
Fig. 4 ¹H-NMR spectra of the aliphatic part of glibornuride in the presence of different concentrations of β-CyD
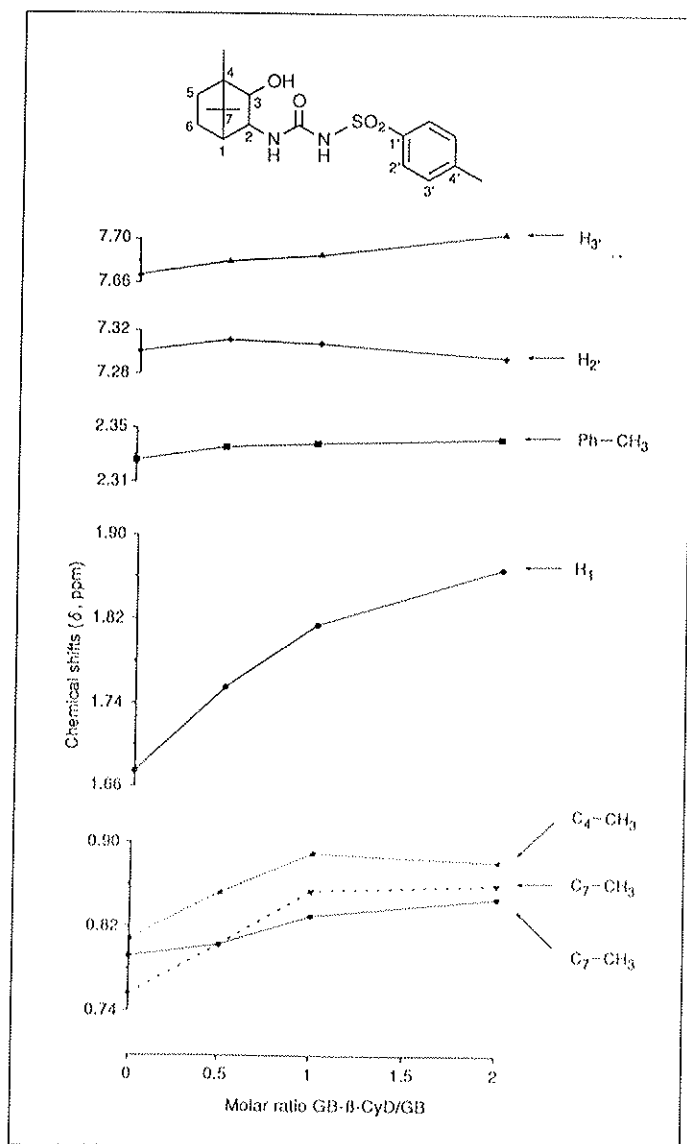

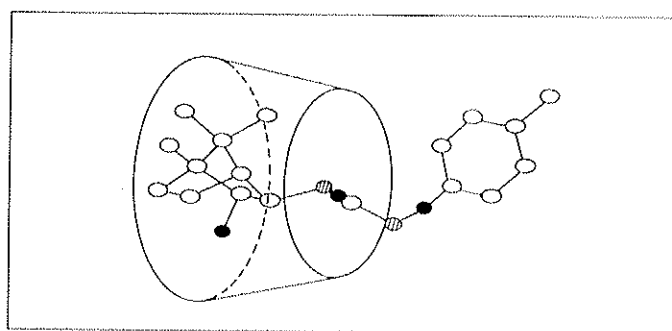Fig. 5 Chemical shifts of protons of glibornuride in solutions varying in β-CyD concentration

Fig. 6 Structure for the glibornuride-β-CyD* inclusion complex in aqueous solution concluded from ¹H-NMR studies
* The geometry of the drug was optimized through a simple minimization procedure (MM-2 calculations)

¹H-NMR studies: structure of the GB/β-CyD inclusion complex in solution. The insertion of a guest molecule into the CyD cavity is clearly reflected in changes in ¹H-NMR chemical shift values (10, 11). The effects of GB at different concentrations on the ¹H-NMR spectrum of β-CyD are shown in Fig. 3. The H_5 signal, although partially obscured by H_3 and H_6 signals, appeared to shift upfield with increasing GB concentration. At a 1 : 1 GB : β-CyD molar ratio a well-defined doublet, assigned to H_5, is apparent. Considering the difference in relative magnitude of $\Delta\delta$ between H_3 and H_5, it can be assumed that the association between GB and β-CyD involves an introduction of the drug molecule from the secondary hydroxyl side of the β-CyD; this is not the case with tolbutamide and clorpropamide/β-CyD complexes (10) and barbiturate/β-CyD (11).

The H_1, H_2 and especially H_4 signals shifted downfield with increasing GB concentration (Fig. 3). This indicates that the drug interacts with the more external part of the CyD cavity, particularly when it is present in excess (22).

The effects of β-CyD at different concentrations on the ¹H-NMR spectrum of GB were more difficult to interpret since some signals (especially those of protons in the aliphatic part of the molecule) could not be assigned. The region δ = 1.7–0.6 ppm shows marked changes as the concentration of CyD is increased (Fig. 4). All signals shifted downfield with increasing β-CyD concentration (Fig. 5). Similar patterns have been reported for other drug-CyD systems (10, 11, 22). The changes undergone by the protons of the aliphatic methyls (C_4–CH_3; C_7–CH_3; C_7–CH_3) and by the C_1-H proton are apparently greater than those corresponding to the p-tolyl group, which suggests that it is the bornyl part of GB that interacts most strongly with, and is encapsulated by the CyD molecule. The changes in chemical shift for these protons (Fig. 5) are in keeping with a 1 : 1 molar ratio in the GB/β-CyD complex (23).

A possible model of the GB/β-CyD inclusion complex at pH 12 is shown in Fig. 6.

Complexation in the solid state. Spectrophotometry indicated that the GB/β-CyD complex obtained by coprecipitation had 1 : 1 stoicheiometry, which is in keeping with the NMR results. A GB : β-CyD molar ratio of 1 : 1 was thus used when preparing the complex by the freeze-drying method.

Significantly different X-ray diffraction patterns are to be expected if an inclusion complex is formed, since crystal structure will change (21, 24). X-ray diffractograms corresponding to the 1 : 1 GB/β-CyD complexes and to a simple 1 : 1 physical mixture are shown in Fig. 7.

Freeze-drying of the pure drug did not afford a completely amorphous product, whereas the inclusion complexes obtained
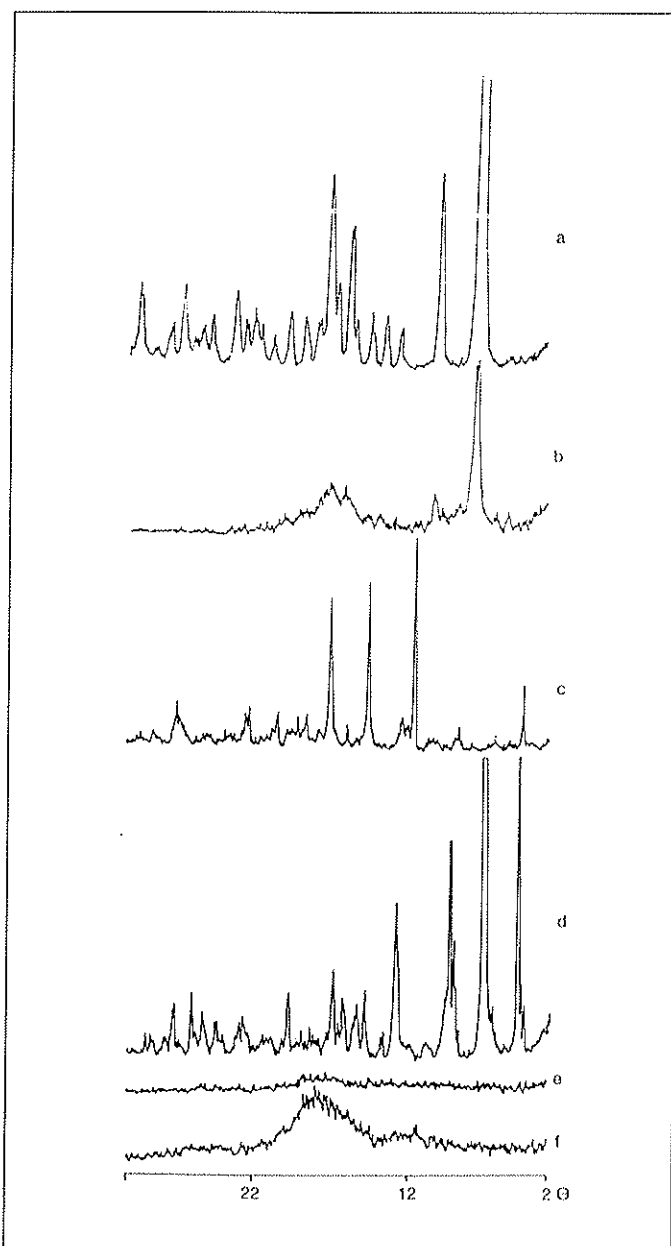
Fig. 7 X-Ray diffractogram corresponding to: a) GB, b) Freeze-dried GB, c) β-CyD, d) Physical mixture GB/β-CyD (1 : 1), e) GB-β-CyD complex coprecipitated, f) GB-β-CyD complex freeze-dried

Fig. 8 Thermogram corresponding to: a) GB, b) Freeze-dried GB, c) β-CyD, d) Physical mixture GB/β-CyD (1 : 1), e) GB-β-CyD complex coprecipitated, f) GB-β-CyD complex freeze-dried

by both methods show peaks with very low intensity. This indicates that both inclusion complexes are markedly less crystalline than either the physical mixture or the individual components. The findings for the complex obtained by freeze-drying are in keeping with those of Oguchi et al. (25) for drug-additive binary systems obtained by a similar method. We have previously reported some crystallinity of a GB/β-CyD complex obtained by coprecipitation (8); however in that study water, rather than an acid buffer, was used as the coprecipitation medium.

DSC thermograms are shown in Fig. 8. The endothermic peak of pure GB at 197 °C is present in the thermogram of the physical mixture but absent from those of the complexes, providing further evidence that the solid complex has a structure which is clearly distinct from that of the physical mixture (21).

Dissolution behaviour of 1 : 1 GB/β-CyD complexes. The dissolution profiles of GB, the 1 : 1 physical mixture and the inclusion complexes are show in Fig. 9. The dissolution of the drug is solubility-limited after 60 min in all the formulations assayed. Both

Table 1 Values of dissolution efficiency at 0 – 120 min obtained for the formulations studied
GB glibornuride. GB_{FD}: freeze-dried glibornuride. PM: Physical mixture GB/β-CyD (1 : 1). C_{COP}: GB-β-CyD complex (1 : 1) obtained by coprecipitation. C_{FD}: GB-β-CyD complex (1 : 1) obtained by freeze-drying

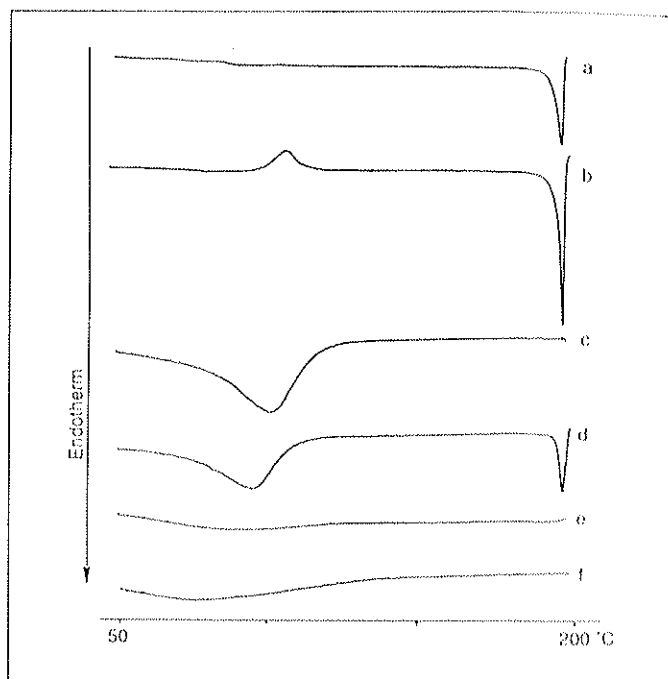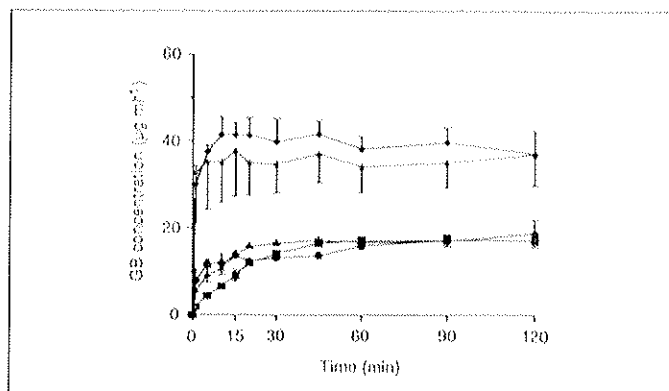|	Dissolution Efficiency ($\times\ 10^{-4}$)				
	GB	GB_{FD}	PM	C_{COP}	C_{FD}
	1.69	1.97	1.74	5.10	4.97
	1.82	2.02	1.90	3.57	4.82
	1.86	1.83	1.86	4.00	4.32
X	1.79	1.94	1.83	4.22	4.70
S.D.	0.09	0.09	0.06	0.79	0.34

Fig. 9 Dissolution profiles of GB and its inclusion complexes
■ GB; ▲ Freeze-dried GB; ● Physical mixture GB/β-CyD (1 : 1); ◆ GB-β-CyD complex (1 : 1 coprecipitate); ✳ GB-β-CyD complex (1 : 1 freeze-dried)

complexes dissolved more rapidly than either GB itself or the physical mixture. Dissolution efficiencies (0–120 min) are shown in Table 1. One-way analysis of variance in dissolution efficiency (0–120 min) revealed significant differences between the complexed and noncomplexed formulations ($F_{(4,10)} = 40.77$, $\alpha < 0.01$).

The LSD test grouped the formulations as follows:

GB GB (freeze-dried) Physical mixture (1 : 1)

Complex (coprecipitate) Complex (freeze-dried)

Thus no increase in the dissolution efficiency of GB was found either after freeze-drying or after simple mixing with β-CyD. However, the formulation as an inclusion complex with β-CyD (whether by coprecipitation or freeze-drying) significantly increased dissolution efficiency.

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