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# Preparation and Physico-Chemical Study of Inclusion Complexes between Idebenone and Modified β-Cyclodextrins

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**Abstract.** The inclusion properties of modified β-cyclodextrins (trimethyl-β-cyclodextrin, dimethyl-β-cyclodextrin and hydroxypropyl-β-cyclodextrin) towards idebenone were compared with natural β-cyclodextrin. The inclusion complexes were prepared by different methods (coprecipitation, kneading, and freeze-drying) and characterized by differential scanning calorimetry, X-ray diffractometry, UV, CD and NMR spectroscopy. The results obtained by CD and NMR spectroscopy indicate a different orientation of idebenone in dimethyl-β-cyclodextrin with respect to other cyclodextrins. Stability constants of the complexes were determined in water at various temperatures and consequently thermodynamic parameters were obtained. All cyclodextrins are able to significantly increase the water solubility of idebenone, particularly dimethyl-β-cyclodextrin and hydroxypropyl-β-cyclodextrin, as a result of complexation. Consequently, they enhance the dissolution rate of the complexed drug compared to the free drug.

**Key words:** Cyclodextrins, idebenone, characterization of complexes, dissolution study.

## 1. Introduction

Idebenone (6 - (10 - hydroxydecyl) - 2,3 - dimethoxy - 5 - methyl - 1,4 - benzoquinone) (IDE; see Table II for structure) is a recently synthesized benzoquinone derivative that has been shown to have beneficial effects for neurological disorders of both vascular and degenerative origins [1–3].

The drug protects mitochondrial membranes against lipid peroxidation by neutralizing free radicals and limiting their production at the level of the mitochondrial

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respiratory chain [4]. It is able to restore normal ATP and lactate levels in the brain and to normalize the glucose metabolism, which are seriously compromised during cerebral accidents [5, 6]. Furthermore, IDE inhibits the decrease of acetylcholine [7] and accelerates the turnover of serotonin [8] in the brain of rats affected by cerebral ischemia, improving the mechanisms of memory and learning.

IDE has a highly lipophilic structure, hence it is very poorly soluble in water. The only pharmaceutical form present today is a solid for oral administration. Following administration in man, the hematic peak level is observed after 1 or 2 h [9]. IDE was rapidly eliminated and no accumulation was observed [10].

It is well known that the increase of water solubility of drugs can lead to a more rapid and full absorption after oral administration.

During the past decades many methods have been described to improve the dissolution rate of poorly water soluble drugs from solid dosage forms, particularly the use of solid dispersion systems with water soluble agents, such as polyvinylpyrrolidone [11], chitin or chitosan [12], and microcrystalline cellulose [13]. However, amorphous drugs in a soluble matrix can often crystallize, resulting in inferior dissolution and bioavailability due to this increase in crystallinity [14].

Cyclodextrins (Cyds) are cyclic oligosaccharides able to form inclusion complexes with non polar substances. They can be used to modify the physico-chemical characteristics of many drugs such as solubility, dissolution rate, bioavailability and to reduce some undesired effects [15–17]. For these reasons they could be candidates for novel drug carriers [18, 19]. A natural $\beta$-cyclodextrin ($\beta$-Cyd) was the most useful for these purposes because the dimensions of its cavity are comparable with those of most drugs, but its low water solubility limited the solubility of the complex itself. In recents years, therefore, modified Cyds have been investigated, because they are highly soluble both in water and organic solvents. Moreover, they are able to form inclusion compounds in the same way as parent Cyds [20, 21].

In a previous paper [22] we described the preparation and characterization, in the solution and solid states, of an inclusion complex between IDE and $\beta$-Cyd. The complexation increased the water solubility and dissolution rate of IDE. Due to the nefrotoxicity of $\beta$-Cyd [23] it is not possible to use this complex for parenteral administration. For this purpose hydroxypropylated-$\beta$-Cyd can be used [19].

In this paper the preparation of inclusion complexes of IDE with modified Cyds (2,6-di-*O*-methyl-$\beta$-Cyd, 2,3,6-tri-*O*-methyl-$\beta$-Cyd and 2-hydroxypropyl-$\beta$-Cyd) are described. The complexes obtained were characterized in the solid state by differential scanning calorimetry (DSC) and X-ray diffractometry, and in solution by UV, CD and NMR spectroscopy. IDE solubility in the presence of modified Cyds and the dissolution rate of free and complexed IDE were evaluated with respect to the IDE-$\beta$-Cyd complex.

## 2. Experimental

### 2.1. MATERIALS

Idebenone (IDE) was kindly provided by Takeda Italia Farmaceutici, S.p.A. (Roma, Italy) and used without further purification. β-Cyclodextrin (β-Cyd) and hydroxypropyl-β-cyclodextrin (HP-β-Cyd), with 0.6 degree of average substitution, were provided by SPAD S.p.A. (Cassano Spinola, Italy). Heptakis-(2,6-di-*O*-methyl)-β-cyclodextrin (DM-β-Cyd) and heptakis-(2,3,6-tri-*O*-methyl)-β-cyclodextrin (TM-β-Cyd) were supplied by Nikon Skoduhhin Kako Co., Ltd (Tokyo, Japan).

All other chemicals and solvents were of analytical reagent grade. Double distilled water was used.

### 2.2. PREPARATION OF IDE–CYD COMPLEXES

The solid complex of IDE and β-Cyd was prepared by two different methods, coprecipitation and freeze-drying, as described in our previous paper [22].

The IDE-DM-β-Cyd complex was prepared by the kneading, coprecipitation and freeze-dried methods:

*Kneading.* A mixture of DM-β-Cyd and IDE (1 : 1 mole ratio) was wetted in methanol : water (4 : 6 v/v) solution and kneaded thoroughly for 60 min. During this process an appropriate quantity of the solvent mixture was added. The paste was dried under reduced pressure at room temperature for 1 day.

*Coprecipitation.* DM-β-Cyd (2 g, $15 \times 10^{-4}$ mol) was solubilized at room temperature in 15 mL of water, then IDE (0.255 g; $7.5 \times 10^{-4}$ mol) was added with stirring. The solution obtained was heated at 40 °C and immediately an orange precipitate was observed. It was separated from the solution and dried under reduced pressure at room temperature.

*Freeze-drying.* DM-β-Cyd (1 g, $7.7 \times 10^{-4}$ mol) was solubilized in 50 mL of water at room temperature, then an excess amount of solid IDE was added and the suspension was stirred at room temperature for 1 week, until equilibrium was reached. The suspension was filtered through a 0.45 μm Millipore filter and the filtrate was freeze-dried (Edwards Modulyo 4K).

The IDE–TM-β-Cyd complex was prepared by the kneading (1 : 3 mole ratio) and freeze-drying method with the same procedures described for the IDE-DM-β-Cyd complex. The IDE-HP-β-Cyd complex was obtained by freeze-drying in the same way as the IDE-DM-β-Cyd complex.

*Determination of the composition of the solid IDE-Cyd complexes.* The IDE-Cyd complexes obtained by coprecipitation or freeze-dried methods were analyzed by UV spectroscopy to determine the drug–Cyd molar ratio.

The IDE-CyD complexes (20 mg) were solubilized in 50 mL of methanol and the solution was analyzed by UV spectroscopy at 280 nm to determine the IDE concentration. $\beta$-Cyd, which is not soluble in methanol, was removed by filtration (Millipore 0.5 $\mu$m) before the UV analysis. The amount of Cyds was determined by difference. In this way it was possible to calculate the mole ratio.

### 2.3. Differential scanning calorimetry (DSC)

DSC scans were recorded on a Mettler DSC 12E differential scanning calorimeter equipped with a Haake D8-G cryostat. A Mettler TA89E and FP89 system software was used for data acquisition. IDE alone, the Cyds, the complexes and the physical mixtures were scanned at a speed of 10 °C/min from 30 to 380 °C.

### 2.4. X-ray Diffractometry

X-ray diffraction patterns of powdered samples were recorded using a PW 1050 Powder Diffractometer (Philips). Experimental settings: Ni-filtered Cu radiation ($\lambda$ = 1.5418 Å); tube settings 40 kV, 20 mA; angular speed 1° ($2\theta$) $min^{-1}$; 0–0.1–1 slits.

### 2.5. UV and CD Spectroscopy

UV spectra of IDE alone ($2.3 \times 10^{-5}$ M) and in the presence of Cyds (mole ratio 1 : 1) were recorded in water by a Uvikon 860 spectrophotometer (Kontron). CD spectra of free IDE ($5 \times 10^{-5}$ M) and in the presence of Cyds (mole ratio 1 : 1) were obtained in a methanol : water solution (3 : 7 v/v) with a Jasco J-600D recording spectropolarimeter.

### 2.6. NMR Spectroscopy

$^{1}$H-NMR and $^{13}$C-NMR spectra were measured using a Varian Gemini 300 spectrometer at a probe temperature of 303 K. TMS was used as external reference. The spectrum of free IDE ($2.11 \times 10^{-2}$ and $4.13 \times 10^{-2}$ M, respectively, for $^{1}$H- and $^{13}$C-NMR spectrum) was recorded in $CDCl_3$ while the complexes (saturated solutions) and the Cyds ($10 \times 10^{-2}$ and $43 \times 10^{-2}$ M, respectively, for $^{1}$H- and $^{13}$C-NMR) spectra were recorded in $D_2O$ (0.7 mL).

### 2.7. Solubility Studies

Excess amounts of IDE were added to aqueous solutions containing various concentrations of Cyds (0–$16 \times 10^{-3}$ M and 0–$38 \times 10^{-3}$ M for $\beta$-Cyd and modified Cyds, respectively) [24] and shaken for 1 week at various temperatures (25, 37 and 45 $\pm$ 0.5 °C). The suspensions were filtered through a 0.45 $\mu$m Millipore filter and

a portion of each filtrate was diluted and the IDE concentration was determined by UV spectroscopy at 280 nm. Each experiment was carried out in triplicate.

Apparent 1 : 1 stability constants ($K_c$) were calculated from the straight portion of the phase solubility diagrams according to the following equation [24]:

$$K = \frac{\text{Slope}}{\text{Intercept}\,(1 - \text{Slope})} \tag{1}$$

### 2.8. Dissolution Studies

The dissolution rates of free and complexed IDE were measured according to U.S.P. 23° paddle method in a dissolution apparatus equipped with a UV spectrophotometer model HP 8452A (Hewlett Packard, Cernusco S/N (Mi), Italy). The amount of free IDE (50 mg) or corresponding amounts in IDE–Cyd complexes was suspended in 900 mL of pH 1.1 buffer solution and stirred at 100 rpm at 37 ± 0.5 °C. At fixed time intervals the concentration of IDE in solution was assayed spectrophotometrically at 280 nm. The experiments were carried out in triplicate.

## 3. Results and Discussion

UV analysis of IDE–Cyd complexes prepared by coprecipitation and freeze-dried method indicates the following drug–Cyd molar ratios: 1 : 1 for the coprecipitated IDE–DM-β-Cyd complex, 1 : 2 for the coprecipitated IDE–β-Cyd complex and 1 : 3 for all IDE–Cyd complexes prepared by the freeze-dried method.

The characterization of IDE–Cyd solid complexes was performed using DSC and X-ray diffractometry.

DSC thermograms of the IDE-DM-β-Cyd system are shown in Figure 1. The thermograms of the complex prepared by both methods show the disappearance of the IDE fusion peak at 54 °C, whereas this peak is observed in the physical mixture. Since it was reported [25] that the interaction of Cyds and a drug led to a shift or disappearance of the drug fusion peak, our results allow us to hypothesize an interaction between IDE and DM-β-Cyd. In the thermograms of the complexes an endothermic peak at 225 °C and at 210 °C, for the coprecipitated and the kneaded complex respectively, is also present, probably relating to the fusion of the new solid phase. The exothermal peak observed at 150 °C in the DSC thermogram of the kneaded complex is due to the transition from the amorphous to the crystalline form [26]. Similar trends (disappearance of fusion peak of IDE and appearance of a new endothermic peak) were observed for all other systems (thermograms not reported).

X-ray diffraction patterns of the IDE-Cyd complexes are shown in Figure 2. The patterns of the physical mixtures were the superposition of the patterns of the two components (IDE and Cyds). However the intensity of the peaks of the two starting compounds was related to their fraction in the sample.

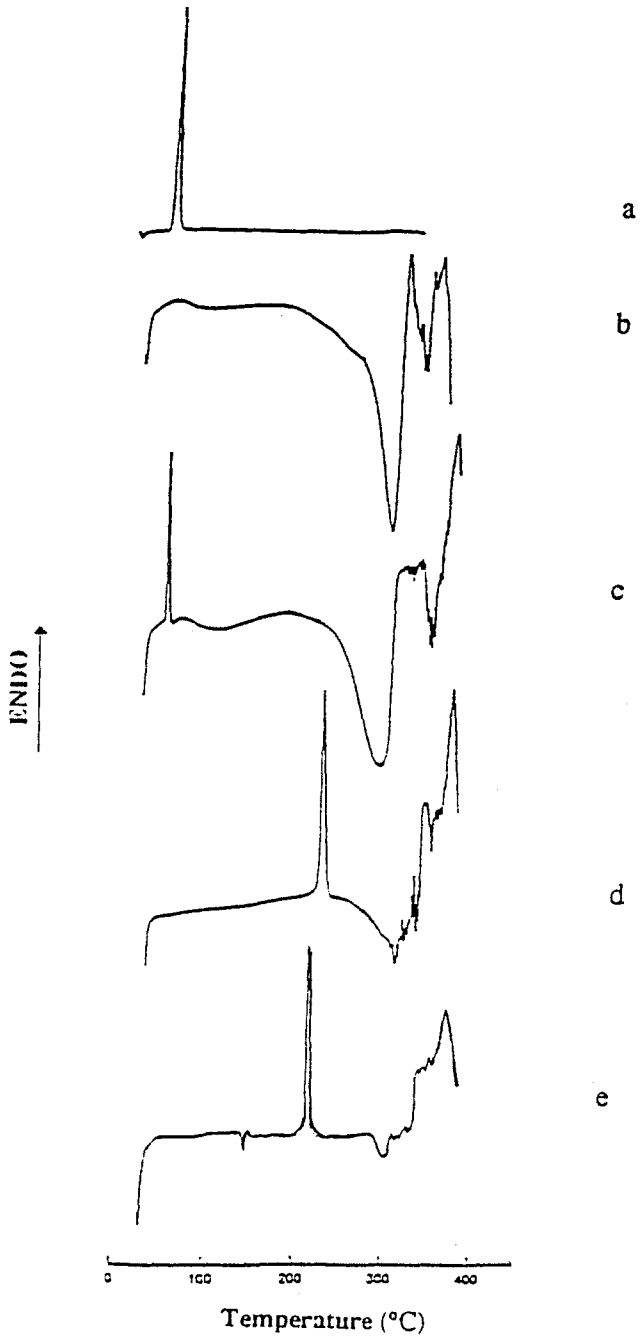



*Figure 1.* DSC thermograms of the IDE-DM-β-Cyd system. (a) IDE; (b) DM-β-Cyd; (c) IDE–DM-β-Cyd physical mixture (1 : 1); (d) IDE–DM-β-Cyd coprecipitated sample; (e) IDE–DM-β-Cyd kneaded sample.

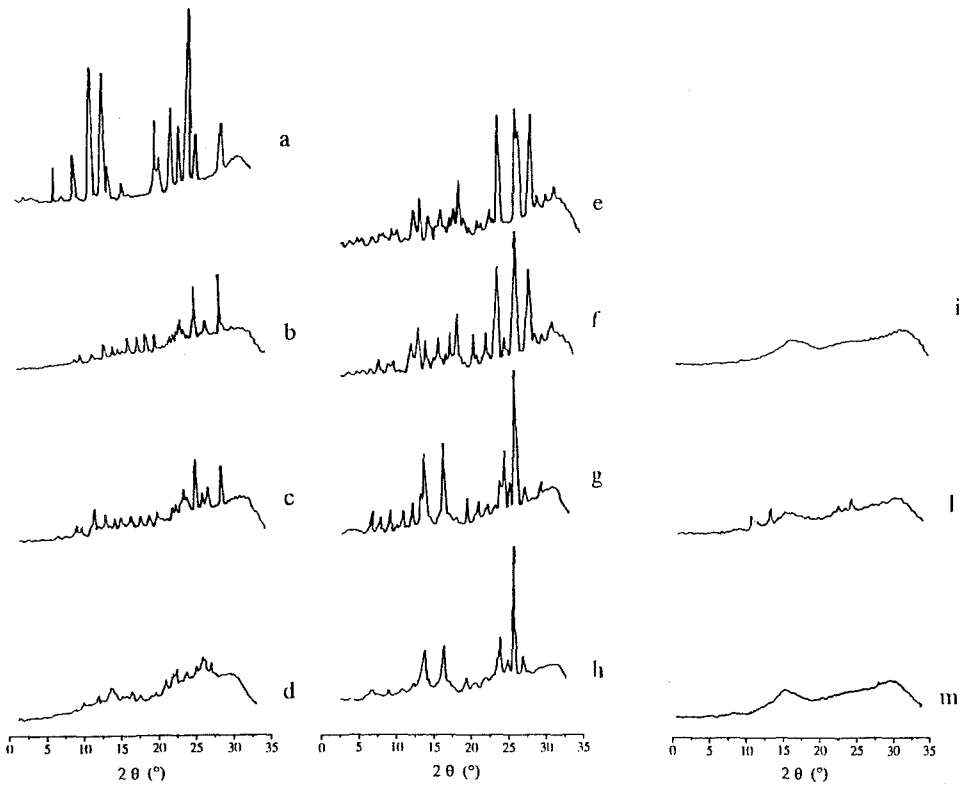



*Figure 2.* X-ray diffraction patterns of IDE-Cyd systems. (a) IDE; (b) TM-β-Cyd; (c) IDE–TM-β-Cyd physical mixture (1 : 3); (d) IDE–TM-β-Cyd complex; (e) DM-β-Cyd; (f) IDE–DM-β-Cyd physical mixture (1 : 1); (g) IDE–DM-β-Cyd coprecipitated complex; (h) IDE–DM-β-Cyd kneaded complex; (i) HP-β-Cyd; (l) IDE–HP-β-Cyd physical mixture (1 : 3); (m) IDE–HP-β-Cyd complex.

The X-ray diffraction pattern of the IDE–Cyd products differed greatly from those of each of the starting compounds and consequently from their physical mixture. This is proof of a new solid phase having a lower crystallinity than the drug and Cyd. The spectra of the coprecipitated and kneaded IDE–DM-β-Cyd products were practically the same, but the spectrum of the kneaded product showed broader and less intense peaks than that of the coprecipitate sample, because of the grinding process used in the preparation procedure.

The diffraction pattern of the IDE–HP-β-Cyd product is similar to that of HP-β-Cyd. However the disappearance of IDE signals, present in the physical mixtures, indicate that IDE interacts with HP-β-Cyd and is transformed into an amorphous compound.

The interaction of IDE with Cyds was further studied in aqueous solution by evaluating the influence of Cyds on spectroscopic characteristics of IDE.

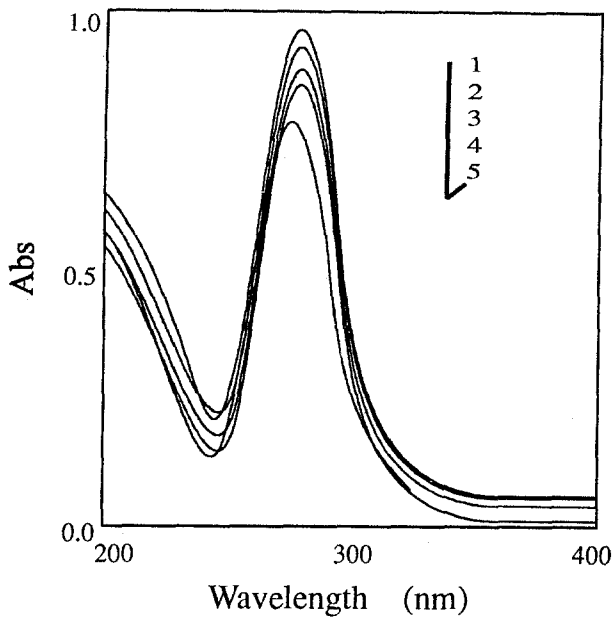



*Figure 3.* UV absorption spectra of IDE alone and in the presence of various Cyds in water. (1) IDE + DM-$\beta$-Cyd; (2) IDE + HP-$\beta$-Cyd; (3) IDE + $\beta$-Cyd; (4) IDE + TM-$\beta$-Cyd; (5) IDE.

IDE shows two UV absorption bands, one at 280 nm due to the $\pi \rightarrow \pi^*$ transition of a conjugate quinone system ($K$ band), and another, with less intensity, in the visible region at 408 nm due to the radical transition of the carbonyl chromophore ($R$ band).

The $K$ band of IDE alone and in the presence of Cyds is shown in Figure 3. The presence of any Cyds produces a small bathochromic shift (1 nm) and an increase of the intensity of this band. This tendency is due to partial shielding of chromophore electrons within the apolar Cyd cavity [27]. In fact, a similar trend was observed when IDE was solubilized in a water/dioxan mixture, which has a low dielectric constant with respect to pure water and hence comparable with the Cyd cavity.

The CD spectra of IDE-Cyd systems are shown in Figure 4. IDE alone shows no appreciable CD band, but in the presence of Cyds a CD band was observed at about 440 nm. This is the result of perturbation of the electronic transition of the drug caused by the asymmetric cavity of Cyds following complexation [28]. The sign of the inducted CD band is related to the spatial position of the asymmetric center and the perturbed chromophore [29]. Hence the negative sign of the CD band of the IDE observed in the presence of DM-$\beta$-Cyd indicates that the drug molecule is arranged in the cavity of this macrocycle in a different way from other Cyds. According to Harata and Uedaira [30], in fact, the negative CD band indicates an

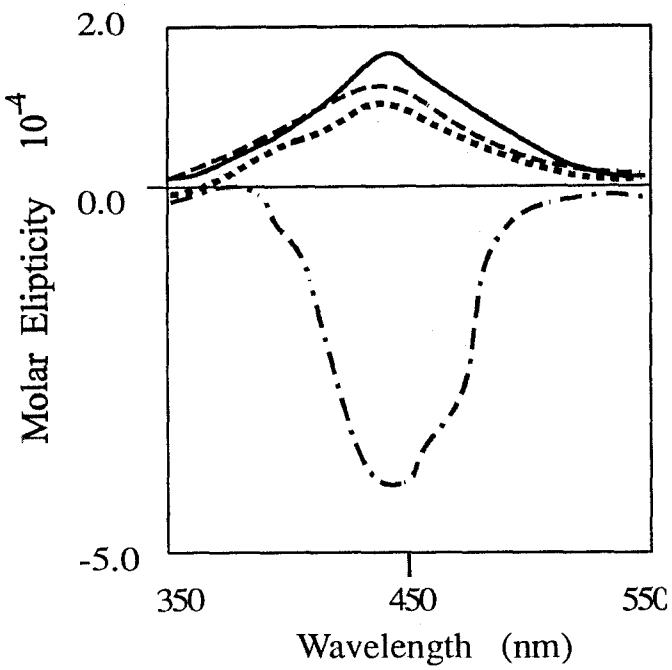



*Figure 4.* CD absorption spectra of IDE in the presence of various Cyds in methanol : water solution. —— IDE + β-Cyd; – – – IDE + HP-β-Cyd; - - - - IDE + TM-β-Cyd; ——— IDE + DM-β-Cyd.

equatorial disposition, while the positive band indicates an axial disposition of the IDE electric dipole moment with respect to the *z*-axis of Cyds.

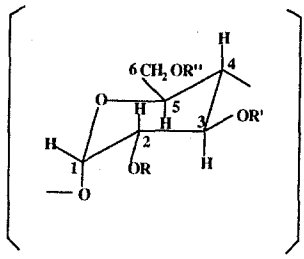
$^1$H-NMR and $^{13}$C-NMR spectroscopy gives direct evidence of the inclusion of IDE in the Cyd cavity.

IDE is almost insoluble in water and it is not possible to obtain its NMR spectrum in $D_2O$; moreover, it is not possible to use $CDCl_3$ as a solvent for all samples, because it causes a desinclusion of the drug from the Cyd cavity. For this reason, the chemical shifts of IDE in the presence of Cyds measured in $D_2O$ were compared with the chemical shifts of free IDE in $CDCl_3$.

Table I shows the effects produced by IDE on the $^1$H chemical shifts of various Cyds. The protons located on the internal surface of the Cyd cavity ($H_3$ and $H_5$) are shifted upfield in the presence of IDE, particularly for DM-β-Cyd. This effect, probably due to a magnetic anisotropy created by the quinone ring, indicates a direct interaction of IDE with the cavity. The $H_6$ protons in DM-β-Cyd (located on the inner surface of the primary hydroxyl group site) are also shifted upfield, indicating that IDE probably penetrates more deeply here than in other Cyds and strongly interacts with this macrocycle.

Table II shows the chemical shifts of IDE alone and in the presence of Cyds. The protons of IDE are shifted, probably, as a result of complexation. DM-β-Cyd

Table I. $^1$H chemical shifts of various Cyds and their displacements in complexes with IDE.

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R=R'=R''=H β-CyD
R=R''=CH ; R'=H DM-β-CyD
R=R'=R''=CH TM-β-CyD

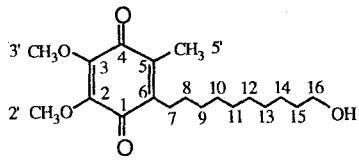
| | β-Cyd | | | DM-β-Cyd | | | TM-β-Cyd | | |
|---|---|---|---|---|---|---|---|---|---|
| H | without | with | Δδ[a] | without | with | Δδ[a] | without | with | Δδ[a] |
| 1 | 5.044 | 5.059 | **+0.015** | 5.215 | 5.198 | **-0.017** | 5.276 | 5.262 | **-0.014** |
| 2 | 3.622 | 3.637 | **+0.015** | — | — | — | — | — | — |
| 3 | 3.942 | 3.910 | **-0.032** | 3.959 | 3.842 | **-0.117** | 3.679 | 3.650 | **-0.029** |
| 4 | 3.559 | 3.587 | **+0.028** | 3.609 | 3.625 | **+0.016** | 3.744 | 3.731 | **-0.013** |
| 5 | 3.821 | 3.785 | **-0.036** | 3.872 | 3.766 | **-0.106** | 3.852 | 3.820 | **-0.032** |
| 6 | 3.854 | 3.853 | **-0.001** | 3.723 | 3.674 | **-0.049** | 3.830 | 3.820 | **-0.010** |
| 2' | | | | 3.545 | 3.558 | **+0.013** | 3.504 | 3.495 | **-0.009** |
| 3' | | | | | | | 3.594 | 3.589 | **-0.005** |
| 6' | | | | 3.376 | 3.366 | **-0.01** | 3.370 | 3.364 | **-0.006** |

a: Δδ = δ complex - δ free.

causes a downfield shift of the methylene protons at $C_7$ of the IDE alkyl chain. These protons are, in the complex, magnetically non-equivalent and give rise to an AB system split further by the coupling with the methylene protons at $C_8$ (Figure 5) and so provide evidence of a strong interaction of IDE with DM-$\beta$-Cyd. Both methyl protons of the quinone ring and the methylene protons at $C_{15}$ of the alkyl chain are also shifted, while the other methylene groups of the chain are less influenced. In the presence of other Cyds, the methylene groups at $C_7$ are shifted downfield, but they are not split into a multiplet. All methylene groups of the IDE alkyl chain are influenced more strongly than DM-$\beta$-Cyd.

$^{13}$C chemical shifts of Cyds in the presence of the drug are shown in Table III. The shifts of $C_1$ observed in all cases are due to a conformational change of Cyd glucosyl residues following interaction with IDE [31] and are correlated with

Table II. $^1$H chemical shifts of free and complexed IDE.




| H | IDE alone | with β-Cyd | Δδ[a] | with DM-β-Cyd | Δδ[a] | with TM-β-Cyd | Δδ[a] |
|---|---|---|---|---|---|---|---|
| 7 | 2.435 | 2.472 | **+0.037** | 2.550-2.467 | — | 2.482 | **+0.047** |
| 8-14 | 1.331 | 1.370 | **+0.039** | 1.327 | **-0.004** | 1.335 | **+0.004** |
| | 1.274 | 1.293 | **+0.019** | 1.250 | **-0.024** | 1.201 | **-0.073** |
| 15 | 1.551 | 1.549 | **-0.002** | 1.473 | **-0.078** | 1.537 | **-0.014** |
| 16 | 3.624 | — | — | — | — | — | — |
| 2'* | 3.976 | 3.965 | **-0.011** | 3.991 | **0.015** | 3.970 | **-0.006** |
| 3'* | 3.979 | 4.006 | **+0.027** | 3.991 | **+0.012** | 3.920 | **-0.059** |
| 5' | 2.000 | 2.033 | **+0.033** | 2.076 | **+0.076** | 2.035 | **+0.035** |

a: Δδ = δ complex - δ free.

*: $^1$H signals may be interchangeble.

thermodynamic parameters. In fact, the more ordered IDE-DM-β-Cyd systems (>$\Delta S$ negatives values) (see Table VI), with respect to β-Cyd and TM-β-Cyd, reflect less conformational flexibility of DM-β-Cyd (higher $^{13}C_1$ shift) when it interacts with IDE.

The shifts of other Cyd carbons are probably due to steric compression interaction with IDE. In the case of DM-β-Cyd, all carbons are significantly influenced, particularly $C_5$ and $C_6$, indicating a deeper interaction of IDE in DM-β-Cyd than TM-β-Cyd and β-Cyd. The higher upfield shift of $C_6$ in DM-β-Cyd is probably due to a close interaction with an electronegative substituent of the host [31].

The $^{13}C$ signals of the guest in the presence of Cyds are generally low because the amount of glucose residues are high compared with that of the drug. A shift of all IDE carbons is, however, evident. The values obtained are reported in Table IV. Higher shifts of the alkyl chain are observed when IDE interacts with TM-β-Cyd, probably because of its narrower cavity with respect to β-Cyd, producing a stronger interaction with the linear chain.

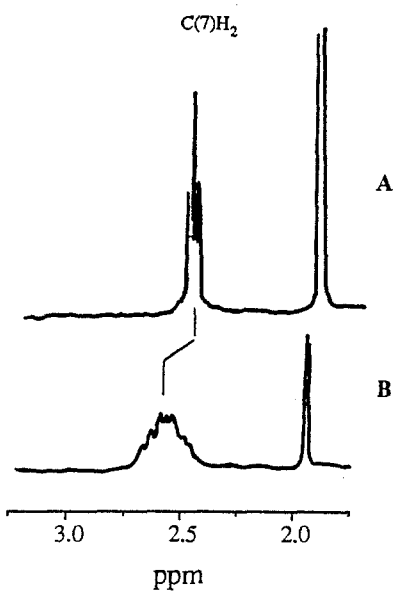



*Figure 5.* Partial $^1$H-NMR spectrum of IDE alone (A) and in the presence of DM-$\beta$-Cyd (B).

### 3.1. SOLUBILITY STUDIES

The influence of various Cyds on the water solubility of IDE was evaluated. The solubility phase diagrams obtained for the drug at 25 °C are of the $A_L$ type [24] (Figure 6), showing a first order dependency of IDE in Cyds. With the increase of temperature (at 45 °C) the solubility curve of the IDE-DM-$\beta$-Cyd system changes to a Bs type (Figure 7) and there is precipitation of the complex. This trend is due to the reduced solubility of DM-$\beta$-Cyd with an increase in temperature [18] which reduces the solubility of the complex formed. UV analysis of the precipitated sample revealed a 1 : 1 mole ratio. All other systems maintained the same trend observed at 25 °C.

Assuming a 1 : 1 stoichiometry for all complexes, the apparent stability constants ($K_c$) were determined from the straight portion of the solubility diagrams. The obtained values are reported in Table V.

The IDE-DM-$\beta$-Cyd complex has, at various temperatures, higher $K_c$ values than other complexes, because the presence of two methyl groups extends the hydrophobic region and increases the interaction with the drug. The methyl group in O(3) present in TM-$\beta$-Cyd increases its lipophilicity with respect to other Cyds. On the other hand the production of a steric hindrance limits the penetration of the benzoquinone ring into its cavity.

Complexation is an exothermal process. In fact, the higher the temperature, the smaller the $K_c$ value and the $\Delta H$ values are always negative (see Table VI). All thermodynamic parameters of the inclusion processes were determined on the basis

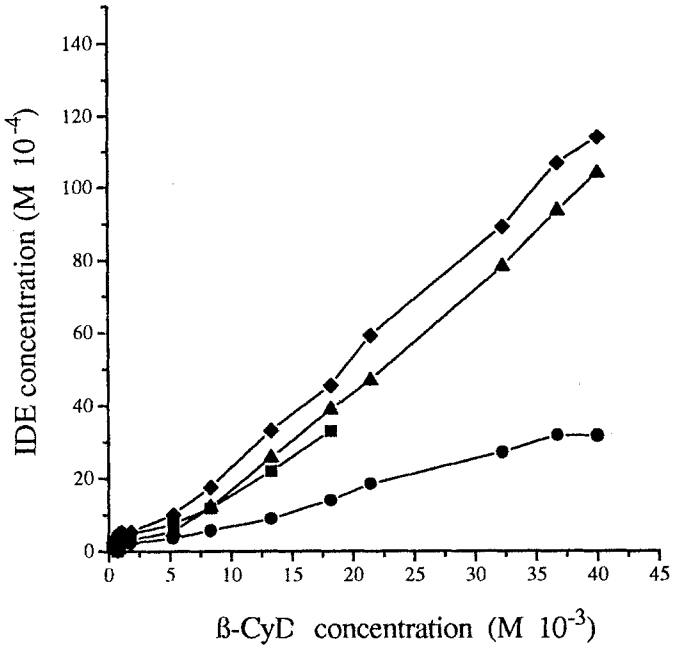



*Figure 6.* Phase solubility diagrams for IDE with various Cyds in water at 25 ± 0.5 °C. ●—● IDE-TM-β-Cyd; ■—■ IDE-β-Cyd; ▲—▲ IDE-HP-β-Cyd; ♦—♦ IDE-DM-β-Cyd.

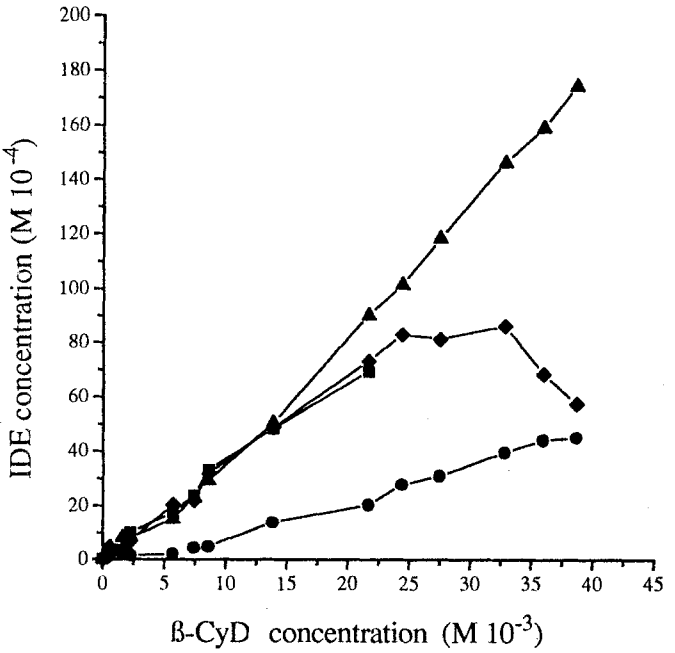
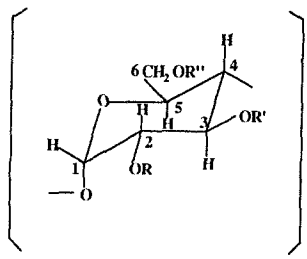



*Figure 7.* Phase solubility diagrams for IDE with various Cyds in water at 45 ± 0.5 °C. ●—● IDE-TM-β-Cyd; ■—■ IDE-β-Cyd; ▲—▲ IDE-HP-β-Cyd; ♦—♦ IDE-DM-β-Cyd.

Table III. Cyd $^{13}C$ chemical shifts and their displacements in the presence of IDE.




R = R' = R'' = H β-CyD
R = R'' = CH ; R' = H DM-β-CyD
R = R' = R'' = CH TM-β-CyD

| | β-Cyd | | | DM-β-Cyd | | | TM-β-Cyd | | |
|---|---|---|---|---|---|---|---|---|---|
| C | without | with | Δδ[a] | without | with | Δδ[a] | without | with | Δδ[a] |
| 1 | 102.861 | 102.991 | **+0.130** | 100.850 | 101.202 | **+0.352** | 98.042 | 98.255 | **+0.213** |
| 2 | 73.115 | 73.115 | **0.000** | 82.491 | 82.556 | **+0.065** | 81.060 | 81.160 | **+0.100** |
| 3 | 74.129 | 74.300 | **+0.171** | 73.601 | 73.965 | **+0.364** | 81.968 | 82.030 | **+0.062** |
| 4 | 82.156 | 82.201 | **-0.045** | 83.181 | 83.610 | **+0.429** | 78.001 | 78.362 | **+0.361** |
| 5 | 72.819 | 72.922 | **+0.103** | 70.970 | 71.810 | **+0.840** | 71.420 | 71.520 | **+0.100** |
| 6 | 61.308 | 61.130 | **-0.178** | 71.740 | 71.021 | **-0.719** | 71.812 | 71.800 | **-0.012** |
| 2' | | | | 59.248 | 59.263 | **+0.015** | 59.140 | 59.140 | **0.000** |
| 3' | | | | | | | 60.770 | 60.901 | **+0.131** |
| 6' | | | | 60.580 | 60.770 | **+0.190** | 59.449 | 59.450 | **+0.001** |

a: Δδ = δ complex - δ free.

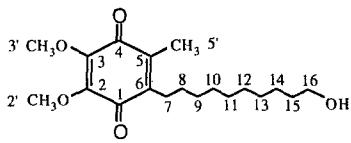
of the dependence of the $K_c$ values on temperature. Complexation is favored by an enthalpic contribution, rather than an entropic one.

The entropy changes in the binding process are due to the disordering, partial or total, of the water layers that surround the complex after binding. These layers are less ordered or contain a smaller number of water molecules than layers before the binding process [32]. The result of this should be a positive $\Delta S$ value.

The binding process between IDE and Cyds occurs with negative $\Delta S$ values (a more ordered system) because other factors can play a relevant role, such as the rigidity of the Cyds and a decrease in the degree of rotational and translational freedom of the drug after complexation.

The enthalpy changes of the binding process are due to the sum of the enthalpies of many processes: (i) release of water molecules from the Cyd cavity; (ii) desolva-

Table IV. $^{13}C$ chemical shifts of IDE in the presence and the absence of various Cyds.




| C | IDE alone | with β-Cyd | Δδ[a] | with DM-β-Cyd | Δδ[a] | with TM-β-Cyd | Δδ[a] |
|---|---|---|---|---|---|---|---|
| 1 | 184.091 | 185.992 | **+1.901** | 184.982 | **+0.891** | 184.822 | **+0.731** |
| 2* | 144.165 | 145.097 | **+0.932** | 144.257 | **+0.092** | 144.400 | **+0.235** |
| 3* | 144.184 | 145.554 | **+1.370** | 145.114 | **+0.930** | 144.532 | **+0.348** |
| 4 | 184.651 | 186.991 | **+2.340** | 185.264 | **+0.613** | 184.921 | **+0.270** |
| 5 | 138.610 | 140.562 | **+1.952** | 140.250 | **+1.640** | 138.880 | **+0.270** |
| 6 | 143.001 | 143.921 | **+0.920** | 143.370 | **+0.369** | 143.760 | **+0.759** |
| 7 | 32.710 | 32.488 | **-0.222** | 33.173 | **+0.463** | 32.965 | **+0.255** |
| 8-15 | 29.740 | 29.660 | **-0.080** | 30.391 | **+0.651** | 30.765 | **+1.025** |
| | 29.473 | 29.420 | **-0.053** | 30.263 | **+0.790** | 30.620 | **+1.147** |
| | 29.331 | 29.391 | **-0.060** | 30.200 | **+0.869** | 30.185 | **+0.854** |
| | 29.272 | 29.325 | **+0.053** | 30.074 | **+0.802** | 29.952 | **+0.680** |
| | 28.650 | 29.290 | **+0.640** | 29.865 | **+1.215** | 27.702 | **-0.948** |
| | 26.330 | 26.690 | **+0.360** | 26.802 | **+0.472** | 27.261 | **+0.931** |
| | 25.667 | 26.150 | **+0.483** | 26.716 | **+1.049** | 26.794 | **+1.127** |
| 16 | 62.940 | 62.728 | **-0.212** | 62.444 | **-0.496** | 62.610 | **-0.330** |
| 2' | 61.151 | 60.813 | **-0.338** | 61.540 | **+0.389** | 61.610 | **+0.459** |
| 3' | 61.151 | 60.681 | **-0.470** | 61.540 | **+0.389** | 61.610 | **+0.459** |
| 5' | 11.880 | 12.960 | **+1.080** | 13.001 | **+1.121** | 13.086 | **+1.206** |

a: $\Delta\delta = \delta$ complex - $\delta$ free .

*: $^{13}C$ signals may be interchangeble.

Table V. Physico-chemical parameters of IDE and its inclusion complexes obtained in water at various temperatures.

| | | Stability constant of complexes (mol/L) | | | |
|---|---|---|---|---|---|
| T (°C) | IDE solubility (mol/L) | IDE-β-Cyd complex | IDE-TM-β-Cyd complex | IDE-DM-β-Cyd complex | IDE-HP-β-Cyd complex |
| 25 | $2.36 \cdot 10^{-5}$ | 5380±96 | 1952±35 | 7934±100 | 5536±47 |
| 37 | $2.60 \cdot 10^{-5}$ | 3524±52 | 1340±89 | 4550±54 | 3220±98 |
| 45 | $2.83 \cdot 10^{-5}$ | 3240±101 | 1104±76 | 3877±10 | 2369±108 |

Table VI. Thermodynamic parameters of the interaction between IDE and Cyds.

| | IDE-β-Cyd complex | IDE-TM-β-Cyd complex | IDE-DM-β-Cyd complex | IDE-HP-β-Cyd complex |
|---|---|---|---|---|
| ΔH | -20.87 | -23.09 | -29.24 | -34.10 |
| ΔS | 1.96 | -13.72 | -22.80 | -41.80 |
| ΔG | -20.46 | -18.07 | -21.13 | -20.25 |

ΔH and ΔG are in KJ $mol^{-1}$ and ΔS is in J $K^{-1}$ $mol^{-1}$.

tion of the drug; (iii) interaction of the drug and Cyds and, (iv) hydrogen bonding between water molecules and the bulk media [33].

Because the energetic contribution of IDE desolvation can be considered the same in all cases, the energetic level of the systems is dependent on the water release from the Cyd cavity. The less favorable $\Delta H$ change in the IDE–$\beta$-Cyd system with respect to the other systems is probably due to stronger interaction of water molecules with free hydroxyl groups, which require more energy to break their bonds.

## 3.2. Dissolution Studies

The dissolution profiles of free and complexed IDE are shown in Figure 8. All complexes dissolve more rapidly than IDE alone, because of the increase of the water solubility and wettability of IDE and of the reduction of molecular crystallinity following complexation. The freeze drying method produces amorphous IDE-Cyd complexes with reduced particle size and consequently they exhibit instantaneous dissolution compared to complexes prepared by other methods.

The IDE–DM-$\beta$-Cyd complex, prepared by the kneading method, dissolves more rapidly than the coprecipitated complex because of its lower crystallinity, as demonstrated by X-ray diffractometry. The lower dissolution profile of the IDE–TM-$\beta$-Cyd complex with respect to other complexes, is probably due to a smaller increase in drug solubility using this macrocycle. Moreover, its lower $K_c$ value with respect to other complexes probably causes the precipitation of the drug after dissolution.

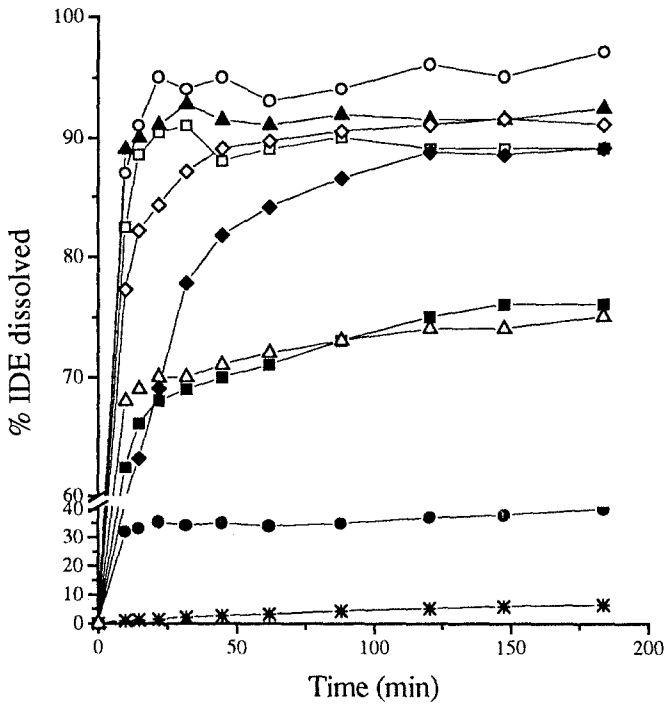



*Figure 8.* Dissolution profiles of free and complexed IDE in buffer solution (pH 1.1) and at 37 ± 0.5 °C. *—* IDE; ●—● IDE-TM-β-Cyd kneaded complex; ■—■ IDE-β-Cyd coprecipitated complex; Δ—Δ IDE-TM-β-Cyd freeze-dried complex; ♦—♦ IDE-DM-β-Cyd coprecipitated complex; ◊—◊ IDE-DM-β-Cyd kneaded complex; □—□ IDE-β-Cyd freeze-dried complex; ▲—▲ IDE-HP-β-Cyd; ○—○ IDE-DM-β-Cyd freeze-dried complex.

## 4. Conclusions

From the results obtained we can conclude that all Cyds used in this study are able to include IDE in their cavity, particularly DM-β-Cyd. CD spectroscopy and NMR studies indicate that the drug is included more deeply and oriented in DM-β-Cyd in a different way compared to other macrocycles.

Complexation significantly increases the aqueous solubility of the drug, particularly in the presence of DM-β-Cyd and HP-β-Cyd. The solubility increase of the drug observed in the presence of HP-β-Cyd can be used to develop a suitable parenteral form.

All IDE-Cyd complexes had a faster dissolution rate than the free drug, in particular, 100% of the freeze-dried complexes dissolve within 15 min. 'In vivo' studies of the complexes are in progress to evaluate the influence of the dissolution increase of IDE on its bioavailability and pharmacological activity.

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