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INCLUSION COMPLEXATION OF 4-BIPHENYLACETIC ACID WITH ß-CYCLODEXTRIN

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ABSTRACT

The preparation of an inclusion complex of 4-biphenylacetic acid (BPAA), a non-steroidal antiinflammatory drug, with ß-cyclo-dextrin is described. The presumible structure of the inclusion system, the molar ratio, which was found 1:1, and the formation constant were calculated by the analysis of IR, UV, DSC, X-ray diffraction, and ^{1}H-NMR. Dissolution rate and solubility were also studied. BPAA solubility in water resulted significantly (4,2-fold) increased by complexation, such

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as its dissolution rate which appears, in the first 12 min, 18 times greater for the complex than the drug alone.

INTRODUCTION

In the last years pharmaceutical modification of drug molecules by inclusion complexation has been extensively developed to improve their dissolution rate (1,2), chemical stability (3,4,5), absorption and bioavailability (6,7). In this respect, an increasing attention in pharmaceutical field have received the cyclodextrins (Cyd) (8,9,10), cyclo-amyloses containing 6 (α-Cyd), 7 (ß-Cyd) or 8 (ϒ -Cyd) α-(1,4) linked D-glucose units, which, for the particular feature of offering hydrophobic cavities associated with a hydrophilic surface (11), permit to form inclusion complexes (a phenomenon called "molecular encapsulation") with various drugs, following the non-covalent interaction of these latter with cyclodextrin atoms lissing the cavity.

4-Biphenylacetic acid (BPAA), a non-steroidal antiinflammatory drug which represents the active metabolite of Fenbufen, is a strong cyclooxigenase inhibitor, as has been proved " in vitro", in homogenates of guinea pig lung (12), and "in vivo", on

experimental prostaglandin-mediated ocular inflammations (13), with a potency greater than indomethacin (14).

Furthermore, BPAA showed a more effective activity than phenylbutazone and acetylsalicilic acid after oral administration.

Such as others NSAIDs, BPAA has also an analgesic activity on the inflammatory pain as well as antihyperthermal effects (15). Finally, it appeared more effective than indometacin, acetylsalicilic acid and phenylbutazone in reducing UV irradiation skin erythema in guinea pig (15).

In the present work the interaction of ß-Cyd with BPAA is investigated, with the aim at improving the aqueous solubility of the acid, so that it may be possible to extend its terapeutic employments and reduce its slight local irritant effect.

MATERIALS AND METHODS

Materials: 4-Biphenylacetic acid was obtained from Janssen (Belgium) (analytical grade) and was recrystallized from ethanol. ß-Cyclodextrin was purchased from Fluka (Switzerland) and was used after recrystallization from water and drying with P_2O_5 in vacuo. All other materials and solvents were of analytical grade, and deionized double distilled water was used.

Solubility studies

The solubility studies were carried out according to the method of Higuchi and Connors (16). Solutions containing various concentrations of ß-Cyd were added of excess amounts of the drug and were shaken at 30°C for 24 hours. After equilibrium for one day more, solutions were pipetted through a cotton filter. A portion of the sample was analyzed spectrophotometrically at 254 nm. The presence of trace amounts of ß-Cyd did not interfere with the assay.

Partition coefficient

Ten milliliters of n-octanol containing 20 mg of BPAA was added to 10 ml of water in a screw-capped tube, which was then shaken at 25°C for 24 hours. The water and organic phases were separed and analyzed spectrophotometrically, at 254 nm, for the drug concentration. PC was defined as the equilibrium concentration ratio in the organic phase to that in the aqueous phase.

Preparation of the solid complex

A solid complex of BPAA and ß-Cyd was prepared by omogeneus coprecipitation method. 0.02 moles of ß-Cyd (22.70 g) were dissolved in water at 50°C and a solution of 0.01 moles of BPAA (2.12 g) in methanol was added. The mixture was stored in a

costant-temperature water bath at 60°C for 1 h, and then stirred for 24 h at room temperature. The complex which precipitated as a microcrystalline powder, was filtered and dried under a vacuum at 50°C for 24 h.

Chemicals analyses were carried out to confirm the stoichiometry of the inclusion system.

Infrared and ultraviolet spectroscopy

IR-spectra were obtained using a Perkin-Elmer IR-spectrophotometer mod. 281, in potassium bromide dischs. UV-spectra were performed on a Perkin-Elmer 330 spectrophotometer equipped with a 3600 data station.

Differenzial Scanning Calorimetry (DSC)

DSC scans were recorded on a Mettler TA 3000 system equipped with TA 10A processor, IBM XT series computer for the evaluation of the data and nitrogen as the purging gas. The sample size were about 12 mg for ß-Cyd, BPAA and inclusion complex. The scannig rate was 2°C/min.

X-Ray Diffraction spectroscopy

Powder X-ray diffractometry was carried out using a Philips PW 1050 diffractometer with a filter Ni CuK(α) radiation detector, at a scannig rate of 1° 2θ/min.

Nuclear Magnetic Resonance (NMR) studies

^{1}H-NMR spectra were measured by a Bruker FT-WP 80 spectrometer at probe temperature (303°K), TMS was used as external reference. The spectra were performed in D_2O and 0.1M NaOD in D_2O.

Dissolution rate studies

Tests were performed using an U.S.P. dissolution apparatus (paddle method) in 900 ml of water as the dissolution medium. The stirring rate was 100 rpm and temperature was mantained at 37°±0.5°C. At appropiate intervals, 3 ml of solution was sampled, filtered through a 0.45 μm membrane filter and diluted with water. Concentration of BPAA was determined using an UV spectrophotometer at 254 nm.

RESULTS AND DISCUSSION

a) Characterization of the complex in solid state

IR spectra of BPAA alone, ß-Cyd, phisical mixture, and complex are showed in Fig. 1.

In the case of the mixture, a simple superimposition of the pure drug and ß-Cyd spectra could be noticed; lower intensity of the carboxylic stretching band at 1690 cm^{-1} may be due to the effect of dilution of the BPAA by means of ß-Cyd.

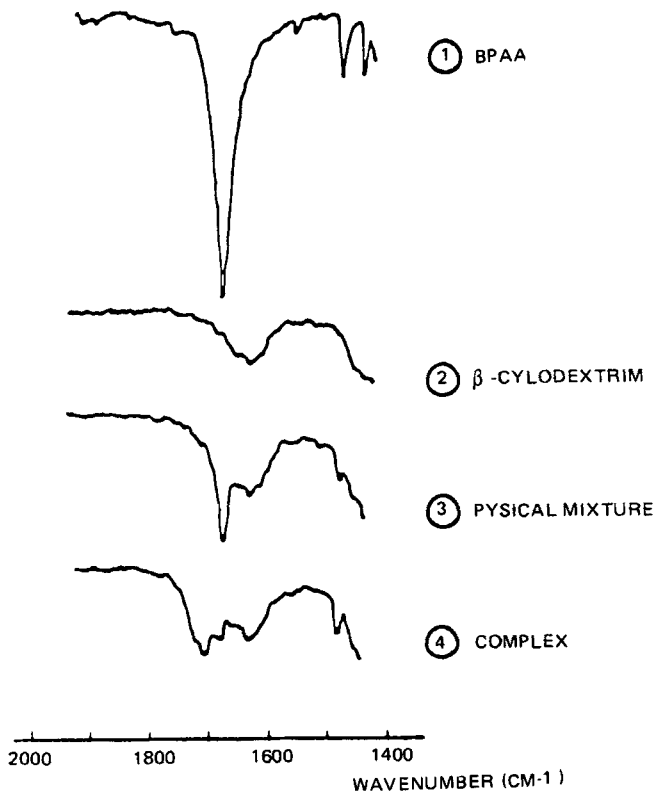

FIGURE 1

IR spectra of: (1) BPAA alone; (2) ß-Cyd-alone; (3) physical mixture of BPAA and ß-Cyd (1:2 in mole ratio); (4) the complex of BPAA with ß-Cyd.

Furthermore, IR data confirm that inclusion between drug and ß-Cyd occurred. In fact, the band at 1690 cm^{-1}, distinctive of BPAA carbonyl stretching, is preserved in the physical mixture, whereas it appears as a lesser intensity, shifted to 1710 cm^{-1} band, in the inclusion complex sample.

The observed behaviour may due to the formation of intramolecular hydrogen bonding between the secondary hydroxyl groups in ß-Cyd and the C=O of the drug (17).

A possible explanation for the noticeable decrease of the carbonyl absorption band intensity may be given by the restriction of C=O into the cyclodextrin cavities (18).

More evidence for the complex formation was given from the differential scanning calorimetry (DSC) thermograms.

Fig. 2 shows the DSC curves of BPAA (1), ß-Cyd (2), physical mixture (3) and complexes between the drug and ß-Cyd deriving from mixing the two components, in homogeneus phase, in 1:1 (4), 1:2 (5) and 1:3 (6) mole ratios, respectively.

The endothermic peak at 161°C related to BPAA, which was also observed in the physical mixture disappeared in the different inclusion complex sistems.

In the complexes, the peak at 100°C related to ß-Cyd and the peak of fusion at 320°C are shifted at lower temperatures (95°C and 310°C respectively). In the meantime the fusion entalpy decreases. The peak at 310°C (curve 4) is made up by the overlap of three peaks, probabily derived by clasters at different composition, while the curve 5 shows the overlap of two peaks and the curve 6 only one peak.

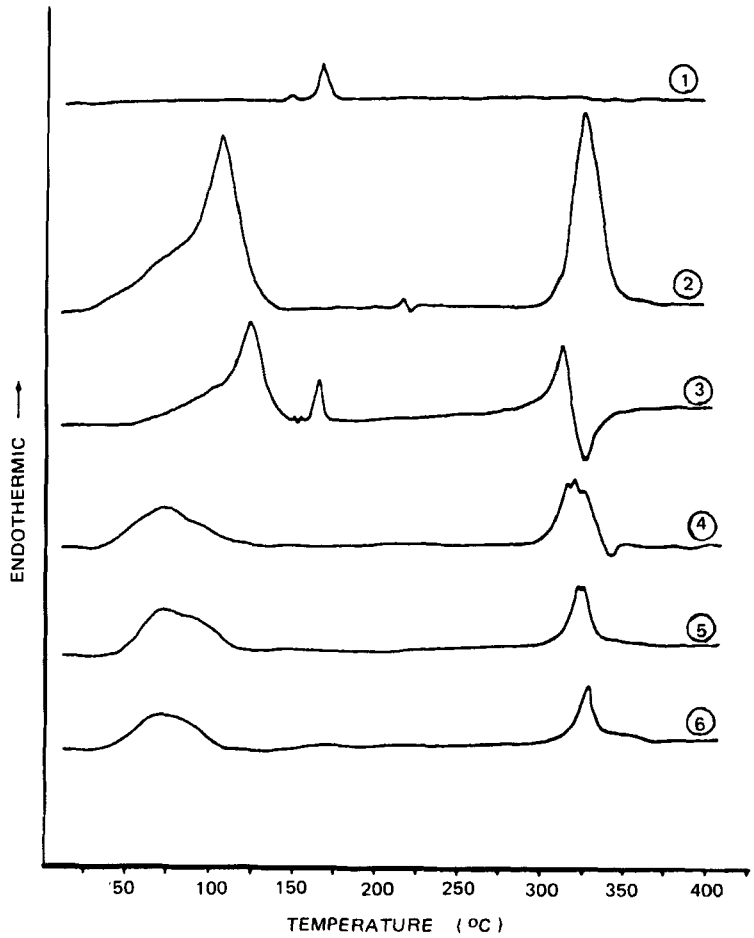

FIGURE 2

Differential scanning calorimetry of: (1) BPAA; (2) ß-Cyd; (3) physical mixture of BPAA and ß-Cyd (1:2); (4), (5), (6) BPAA-ß-Cyd complexes deriving from starting mixtures in (1:1), (1:2) and (1:3) mole ratios, respectively.

These results suggest that the inclusion complex prepared with a high concentration of ß-Cyd give clasters at more homogeneus composition.

Unfortunately, it is not possible to deconvolute Cp curve for establishing the mole ratio in the clasters.

Fig. 3 shows the powder X-Ray diffraction spectra of BPAA, ß-Cyd, the physical mixture and the inclusion complex. Whereas the spectrum of the physical mixture is simply the superposition of each component, that of the inclusion compound is clearly different, showing that a new solid phase has been formed. BPAA-ß-Cyd complex gave somewhat diffuse diffraction patterns, suggesting that it is much less crystalline than the simply mixed counterpart.

b) Characterization of the complex in aqueous solution

Complex formation of BPAA with ß-Cyd in aqueous solution was investigated by solubility, ^{1}H-NMR and UV studies.

Fig. 4 shows the phase solubility diagram for BPAA-ß-Cyd system in water at 25°C.

The increasing in the solubility of drug through the addition of ß-Cyd thus observed was clearly due to the formation of an inclusion complex. The plateau showed by the plot indicates that an insoluble microcrystalline complex was formed in the solution at high ß-Cyd concentration (upper than $4.7 \cdot 10^3$ mol$\cdot$L^{-1}).

In the solution the following simultaneous equilibria are found:

$$\text{ß-Cyd+BPAA} \overset{k_o}{\rightleftharpoons} [\text{ß-Cyd-BPAA}]_{(aq)} \overset{k_1}{\rightleftharpoons} [\text{ß-Cyd-BPAA}]_{(s)} \quad (1)$$

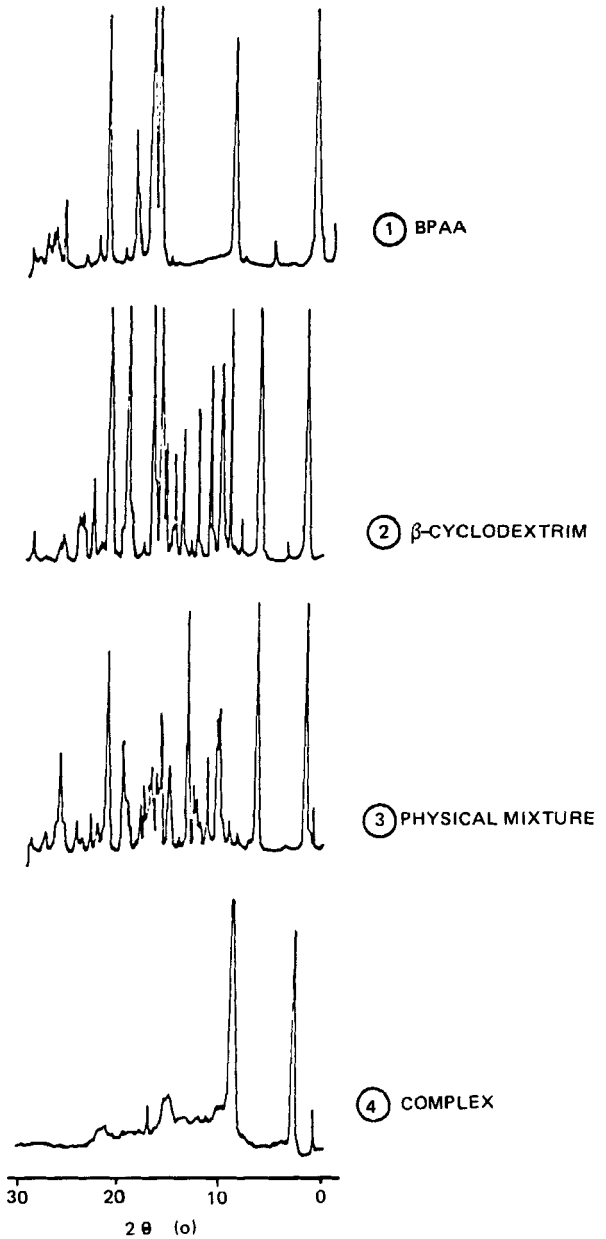

FIGURE 3

Powder X-ray diffraction patterns of: (1) BPAA; (2) ß-Cyd; (3) physical mixture and (4) BPAA-ß-Cyd complex.

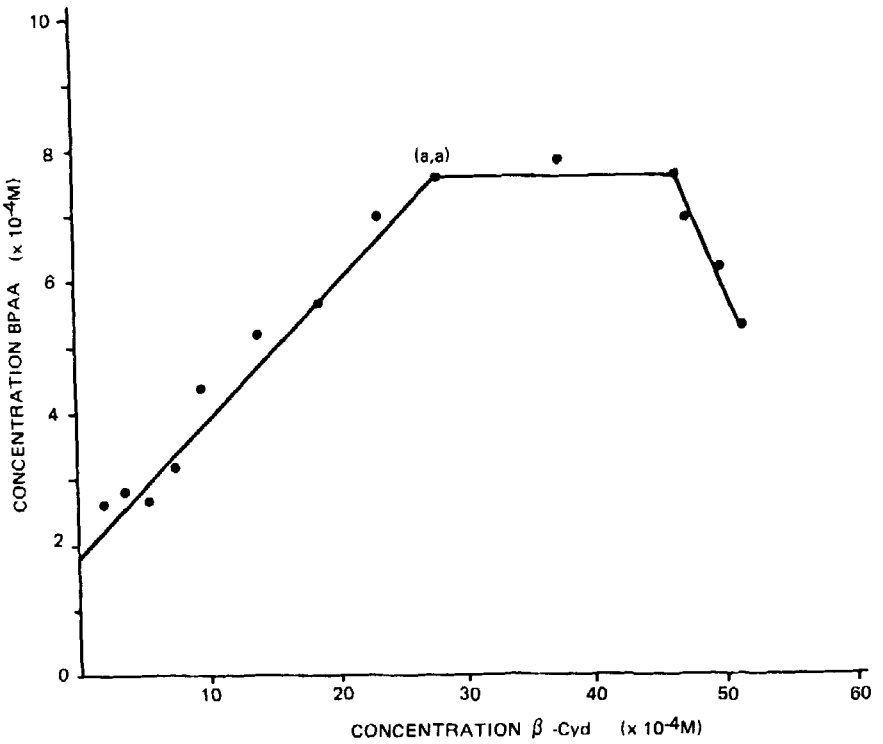

FIGURE 4

Solubility of BPAA as a function of ß-cyclodextrin concentration in water at 25°C.

where (aq) refers to the inclusion complex in water solution (solvated) and (s) means the complex in a solid state.

From the equation (1) we get the formation constant (Ko) for the BPAA-ß-Cyd inclusion complex at a costant temperature, as:

$$K_o = \frac{[\text{ß-Cyd-BPAA}](aq)}{[\text{BPAA}][\text{ß-Cyd}]} \qquad (2)$$

in which [BPAA] is BPAA solubility at that temperature, [ß-Cyd] is ß-Cyd concentration at the point with (a,a) co-ordinates (see Fig. 4) less the inclusion complex concentration in the solution, and $[\text{ß-Cyd-BPAA}]_{(aq)}$ is BPAA concentration at the same co-ordinates less [BPAA], with the assumption that the inclusion complex occurs at a stoichiometric ratio (1:1).

The formation costant for the solid state complex (K_1) has been pointed out as follows:

$$K_1 = \frac{[\text{ß-Cyd-BPAA}]_{(s)}}{[\text{ß-Cyd-BPAA}]_{(aq)}} = \frac{1}{Ko\,[\text{ß-Cyd}][\text{BPAA}]} \qquad (3).$$

In the present study, the found values for the two formation costants are $Ko = 1.93 \cdot 10^3$ $(mol \cdot L^{-1})$ and $K_1 = 1.45 \cdot 10^3$ $(mol \cdot L^{-1})$, respectively.

Fig. 5 reports the dissolution rate profiles of BPAA from ß-Cyd complex and BPAA powders, in water at 37°C.

The two rate costants are $1.12 \cdot 10^{-5}$ and $7.5 \cdot 10^{-5}$ $mol \cdot L^{-1} \cdot sec^{-1}$, respectively. Basing on these results, BPAA appears to be 4.2 times more soluble when it is crowned into ß-Cyd, with an about 18-fold increased dissolution rate in the first 12 min with respect to BPAA alone.

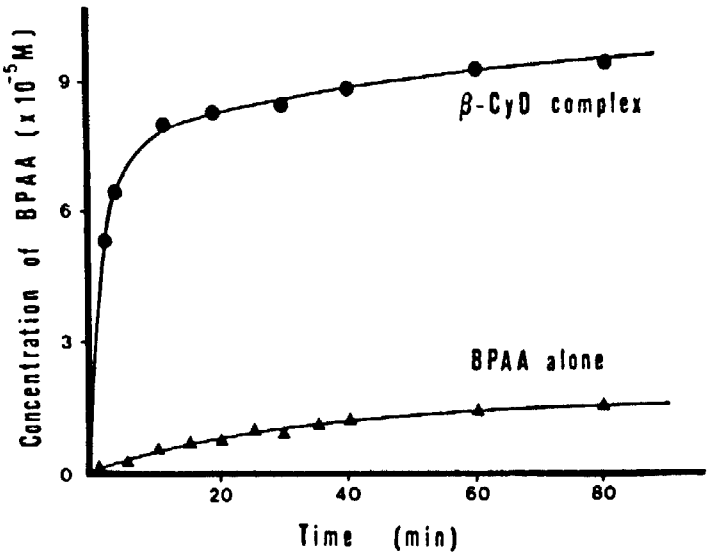

FIGURE 5

Dissolution profiles of: BPAA (▲) and its ß-Cyd complex (●) in water at 37°C, measured by dispersed amount method.

The enhaced dissolution behaviour is probably due to an increased solubility, a reduced cristallinity and a better wettability of the powder drug.

UV-spectra of BPAA and BPAA-ß Cyd complex are illustrated in Fig. 6. No bathochromic shift was observed in the presence of ß-Cyd, while the intensity of the absorption maximum evidently decreased in the complex as a result of a partial shielding of the BPAA chromophore electrons into the cyclodextrin cavity.

c) Presumed Structure of the Inclusion Complex

The ^{1}H-NMR spectrum of BPAA-ß Cyd complex, performed in D_2O as solvent, shows some interesting features. The chemical shift

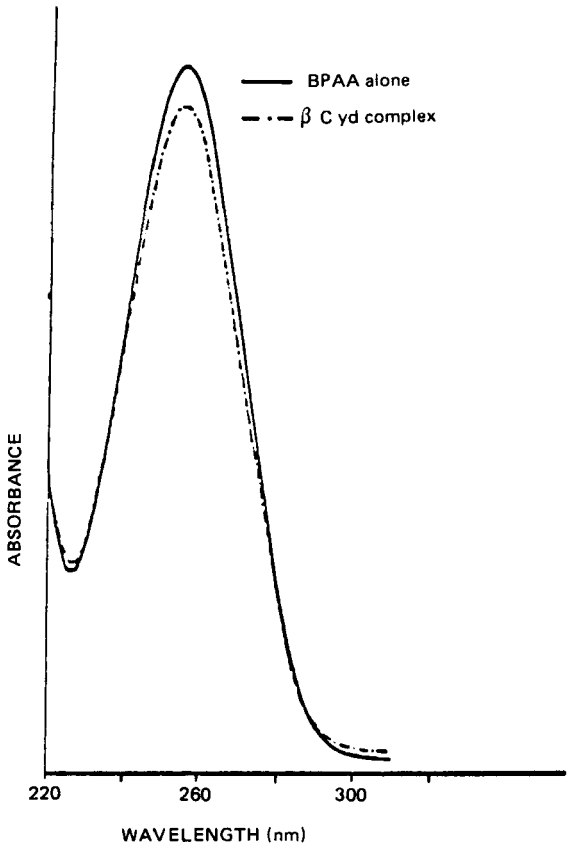

FIGURE 6
Ultraviolet spectra of : BPAA alone (——) and BPAA-ß-Cyd complex (-·-).

changes ($\Delta\delta = \delta_{free} - \delta_{complex}$) for the ß-Cyd protons inside the complex, with respect to the ß-Cyd alone, are as follows:

H_1	H_2	H_3	H_4	H_5	H_6
+0.03	+0.02	+0.15	+0.03	+0.17	+0.05 .

According to the studies of Demarco and Thakkar (19), by these data it is possible to hypothesize that an inclusion occurred

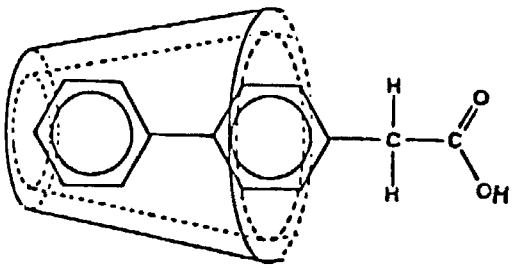

FIGURE 7
Proposed structure of inclusion complex of ß-Cyd with BPAA in aqueous solution.

between the drug and ß-Cyd. Moreover, a broad signal, due to BPAA molecule, is present within the aromatic protons range, suggesting a restricted rotation of the phenyl group owing to the binding with ß-Cyd.

The areas ratio shows that BPAA is present in a molar ratio of about 1:4 with respect to ß-Cyd.

To determinate the effective ratio between the two components in the complex, we performed its ^{1}H-NMR spectrum in a D_2O/0.1M NaOD solution, where the complex is completely soluble. The trend in the chemical shift changes is the same as in the D_2O solution, with an areas ratio confirming the presence of equimolar amounts of ß-Cyd and BPAA in the inclusion complex.

The aromatic system shows again a restriction in the free rotation which suggests that BPAA phenyl moiety is inclused within ß-Cyd cavity.

The chemical shift and aromatic protons behaviours (19) allows us to propose the structure showed in Fig. 7 for BPAA-ß-Cyd inclusion complex in aqueous solution.

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