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COMPARATIVE STUDY ON INCLUSION COMPOUNDS OF 4-BIPHENYLACETIC ACID WITH β-CYCLODEXTRIN, HYDROXYPROPYLATED-β-CYCLODEXTRINS, AND METHYLATED-β-CYCLODEXTRINS

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ABSTRACT

The inclusion behavior of Hydroxypropyl-ß-Cyclodextrin (HP-β-Cyd) and of methylated-β-Cyclodextrins, heptakis-(2,6-di-O-methyl)-β-Cyclodextrin (DM-β-Cyd) and heptakis-(2,3,6-tri-O-methyl)-β-Cyclodextrin (TM-β-Cyd), in solution and solid state was compared with that of natural β-Cyclodextrin (β-Cyd) using an anti-inflammatory drug, 4-biphenylacetic acid (BPAA), as a guest molecule. The solubility of BPAA with β-Cyd and β-Cyd derivatives in aqueous solution were determined. Stability constants were calculated by phase solubility method at various pH values and temperatures. The formation of inclusion complexes with β-Cyd and β-Cyd derivatives in the solid state were confirmed by infrared

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spectroscopy, differential scanning calorimetry and X-Ray diffractometry, and in the liquid phase by ultraviolet spectroscopy, circular dichroism and NMR studies. Dissolution rate and "in vitro" release of BPAA from complexes were examined. The results obtained suggest that DM-β-Cyd is more effective than other β-Cyclodextrins in improving the pharmaceutical properties of BPAA.

INTRODUCTION

Cyclodextrins (Cyds) are cyclic oligosaccharides composed of glucose molecules. They show a remarkable ability to form inclusion complexes with various molecules that fit entirely or partially inside the relatively hydrophobic Cyds cavity (1,2). The inclusion process is mainly influenced by the hydrophobic nature of the interaction between the cavity and the guest molecules, and also by their shape and size (3).

Natural Cyds have been extensively used in many fields, but they are characterized by a relatively low water solubility (1.8% w/v at 25°C for β-Cyd) that limits their application in the pharmaceutical field.

To avoid this problem, considerable attention has recently been paid to chemically modified Cyds, whose physico-chemical properties are largely modified with respect to parent Cyds. For example, the heptakis-(2,6-di-O-methyl)-β-Cyclodextrin (DM-β-Cyd) and heptakis-(2,3,6-tri-O-methyl)-β-Cyclodextrin (TM-β-Cyd) are extremely soluble both in water and organic solvents, less hygroscopic than the parent β-Cyd and more highly surface active (4,5).

Furthermore, β-Cyd derivatives, like natural β-Cyd, can form inclusion complexes which modify the solubility, dissolution rate and bioavailability of the guest molecules (6,7).

NSAIDs are drugs generally characterized by a very poor water solubility. It has been reported that the inclusion of these drugs in β-Cyd enhances solubility and decreases gastric lesive effects (8-10).

In a previous study (11), we described the inclusion complexation in β-Cyd of 4-Biphenylacetic acid (BPAA), a non steroidal anti-inflammatory drug which shows an "in vivo" activity comparable to that of most common anti-inflammatory agents (12,13).

The complexation of BPAA with β-Cyd improved biological activity, gastric tolerability and bioavailability (14). Arima et al. (1990) have reported the inclusion of BPAA in β-Cyd, HP-β-Cyd and DM-β-Cyd and the increase of BPAA delivery through the skin following complexation (15).

Using calorimetric techniques, we have studied the interaction of the β-Cyd-BPAA complex with a lipid model membrane (16).

The aim of this work was to prepare the inclusion complexes of BPAA with natural β-Cyd, DM-β-Cyd, TM-β-Cyd and HP-β-Cyd, to evaluate the effects of β-Cyds (this abbreviation is comprehensive of natural and modified β-Cyd) on BPAA solubility at different pH values and temperature, on dissolution rate and diffusion through an artificial lipid membrane.

Differential scanning calorimetry (DSC), IR spectroscopy and X-Ray diffractometry were used to characterize the complexes in solid state and ^{1}H-, ^{13}C-nuclear magnetic resonance, UV and CD spectroscopy in aqueous solution.

MATERIALS

4-Biphenylacetic acid (BPAA) was obtained from Janssen (Belgium) and recrystallized from ethanol. β-Cyclodextrin (β-Cyd) and Hydroxypropyl-ß-Cyclodextrin (HP-β-Cyd), with

0.6 degree of average substitution, were kindly provided by SPAD (Italia S.p.A.). 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) and used without further purification.

All other chemicals and solvents were analytical reagent grade. Deionized, double-distilled water was used.

METHODS

Preparation of β-Cyds-BPAA complexes

Solid complexes of BPAA with β-Cyd, TM-β-Cyd and DM-β-Cyd were prepared by both freeze-drying and Kneading method.

Freeze-drying method: β-Cyds and BPAA at a 1:1 molar ratio were dissolved in aqueous ammonia solution to increase the solubility of the drug. The solution was stirred for 2h at room temperature and freeze-dried (EDWARDS MODULYO 4K).

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

The HP-β-Cyd-BPAA complex in the solid state was prepared in a 1:1 mole ratio by freeze-drying method only, because the paste produced by the kneading method is sticky and difficult to grind.

Infrared Spectroscopy (IR)

The IR spectra of ß-Cyd derivatives-BPAA complexes were measured as potassium bromide disks on a Perkin-Elmer IR-spectrophotometer mod. 281. The IR spectra of BPAA alone, pure ß-Cyd derivatives and physical mixtures (1:1 mole ratio) were obtained by the same procedures for comparison.

Differential Scanning Calorimetry (DSC)

DSC scans were recorded on a Mettler TA 3000 system equipped with TA 10A processor, with a low temperature cell and nitrogen as the purging gas. Each sample was scanned at a speed of 10°C/min from 30°C to 400°C.

X-Ray diffractometry

X-Ray powder diffraction patterns were collected with a Philips PW 1050 under the following condition: X- ray, Ni-filtered Cu-K_α radiation, scanning rate of 1° 2θ/min.

Solubility studies

Solubility measurements were performed according to Higuchi and Connors (17). Excess amounts of BPAA were added to buffer solutions at different pH values (1.1, 4.0, 7.4) containing various concentrations of β-Cyds and shaken at 37 ± 0.5°C. At equilibrium, after 2 days, an aliquot was filtered through a 0.45 μm Millipore filter. A portion of the sample was diluted and BPAA concentration was determined by UV spectroscopy at 254 nm.

Each experiment was carried out in triplicate.

Apparent 1:1 stability costants (Kc) were calculated from the straight portion of the phase solubility diagrams according to the following equation (17):

$$Kc = \frac{\text{Slope}}{\text{Intercept (1-Slope)}}$$

UV and CD spectroscopy

UV spectra were obtained on a UVIKON 860 spectrophotometer on pH 1.1 buffer solution of BPAA alone and in the presence of different ß-Cyds in a 1:1 mole ratio. CD spectra were obtained with a Jasco J-600D recording spectropolarimeter on the same solutions used for UV spectra.

^{1}H- and ^{13}C-Nuclear Magnetic Reasonance (NMR)

^{1}H-NMR spectra were measured using a Bruker FT-WP 80 spectrometer at probe temperature (303°K), TMS was used as external reference. A Bruker AC 200 spectrometer was used for ^{13}C-NMR spectra. The spectra were performed in D_2O, adding 0.1M NaOD to dissolve BPAA.

Distribution studies in n-octanol/water systems

10 ml of n-octanol, containing 2.5 mg/ml of BPAA, were added to 10 ml of buffer solutions at various pH values (1.1, 4.0 and 7.4) and shaken at 37 ± 0.5°C for 24 h. The concentration of BPAA in the aqueous phase was determined by UV spectroscopy at 254 nm.

The apparent partition coefficient (PC) was determined as the ratio between the total amount of BPAA in the organic and aqueous phases.

Dissolution studies

Dissolution rates of BPAA from the complexes were carried out according to U.S.P. XXI paddle method in 900 ml buffer solutions (pH 1.1, 4.0 and 7.4) as dissolution media. The stirring rate was 100 rpm and temperature was maintained at 37 ± 0.5 °C. At appropriate intervals, 2 ml of solution were sampled and filtered through a 0.45 µm Millipore filter, diluted and assayed spectrophotometrically at 254 nm for BPAA content. 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.

"In vitro" diffusion study

Drug diffusion from different β-Cyds-BPAA complexes with respect to the free drug was studied using a Sartorius SM 16750 absorption simulating apparatus (Membranfilter GmbH, Göttingen, West Germany) (18,19) equipped with a diffusion cell (Sartorius SM 16753). The free BPAA or corresponding amounts of complexes were located in 100 ml of pH 1.1 buffer solution simulating gastric juice (donor phase). The BPAA diffused across an artificial gastric barrier (Sartorius SM 15701, effective area 4.6 cm^2) to 100 ml of pH 7.4 buffer solution simulating plasma (acceptor phase). The experiments were carried out at 37 ± 0.5 °C over 180 min. 2 ml samples were withdrawn at different intervals and the BPAA concentration was determined spectrophotometrically at 254 nm. The values were corrected to compensate for the repeated sampling. The rate of permeation of BPAA through the membrane is expressed by means of the diffusion rate constants (Kd) (20).

RESULTS AND DISCUSSIONS

IR spectroscopy, DSC and X-Ray diffractometry were employed to examine the interaction of BPAA with β-Cyds in the solid state.

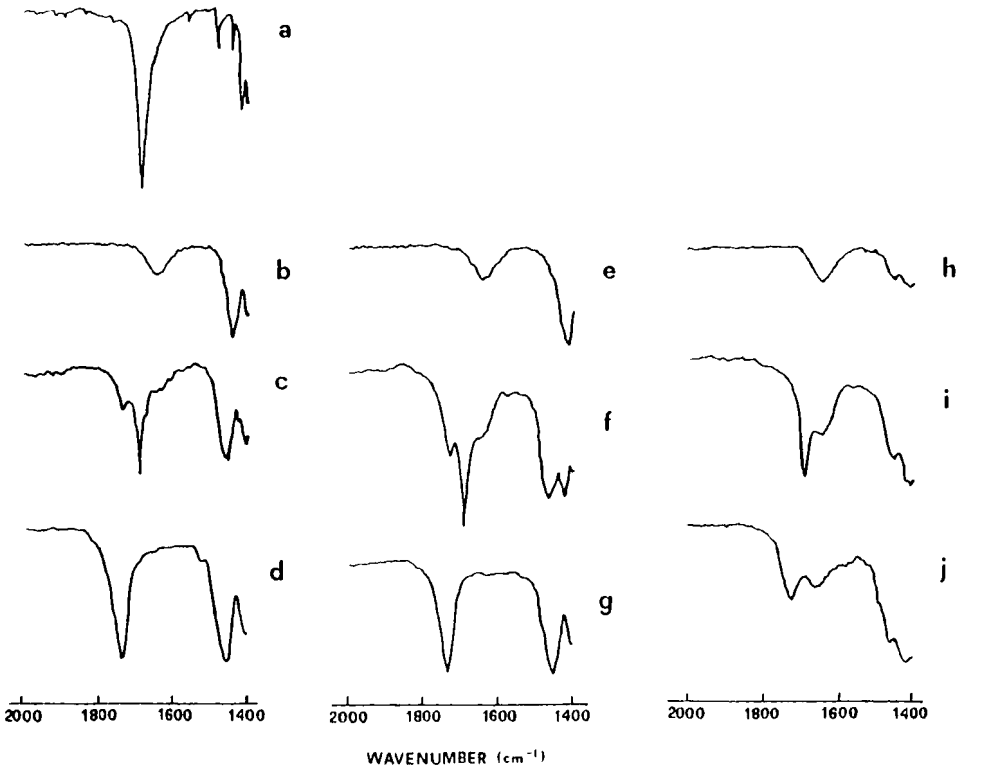


FIGURE 1

IR spectra of β-Cyd derivatives-BPAA systems. a) BPAA; b)TM-β-Cyd; c) TM-β-Cyd-BPAA physical mixture; d) TM-β-Cyd-BPAA complex; e) DM-β-Cyd; f) DM-β-Cyd-BPAA physical mixture; g) DM-β-Cyd-BPAA complex; h) HP-β-Cyd-BPAA; i) HP-β-Cyd-BPAA physical mixture ; j) HP-β-Cyd-BPAA complex.

Fig. 1 shows the IR spectra of BPAA and either methylated- or hydroxypropylated-β-Cyd systems. The β-Cyd-BPAA complex has been characterized in a previous paper (11).

β-Cyd derivatives have no absorption bands in the region of carbonyl stretching vibration near 1700 cm^{-1}.

In the case of the inclusion compounds, there is an evident shift of the stretching band corresponding to BPAA carboxyl (1690 cm^{-1}), to a lower frequency (1730 cm^{-1}), indicating the monomeric dispersion of BPAA due to the interaction with β-Cyd derivatives (21). Conversely, the physical mixtures showed no spectral changes in the absorption bands, but in the TM-β-Cyd- and DM-β-Cyd-BPAA systems a low intensity band is evident at 1730 cm^{-1}, suggesting that a weak interaction occurs between methylated β-Cyd and BPAA during the preparation of KBr disks.

Further evidence of formation of complexes was obtained from the DSC thermograms of BPAA alone and β-Cyd derivatives-BPAA systems shown in fig. 2.

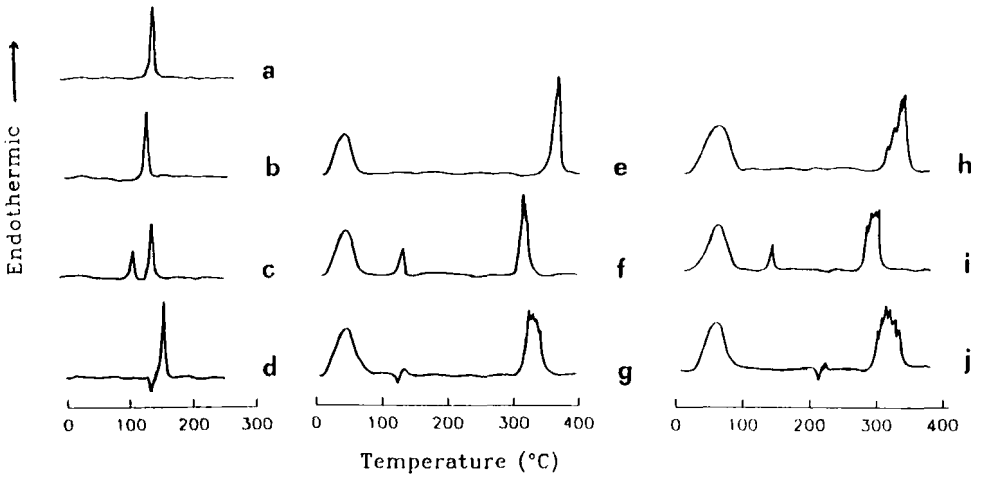


FIGURE 2

DSC thermograms of β-Cyd derivatives-BPAA systems. a) BPAA; b)TM-β-Cyd; c) TM-β-Cyd-BPAA physical mixture; d) TM-β-Cyd-BPAA complex; e) DM-β-Cyd; f) DM-β-Cyd-BPAA physical mixture; g) DM-β-Cyd-BPAA complex; h) HP-β-Cyd-BPAA; i) HP-β-Cyd-BPAA physical mixture ; j) HP-β-Cyd-BPAA complex.

In the thermograms of the inclusion complexes, the melting point of BPAA disappeared and only the melting points of TM-β-Cyd-, DM-β-Cyd- and HP-β-Cyd-BPAA complexes are evident at 174°C, 320-340°C and 310-335°C respectively.

The fusion peaks of DM-β-Cyd- and HP-β-Cyd-BPAA complexes are composed of overlapping peaks, probably due to the formation of inclusion compounds at different stoichiometry (11).

The exothermic peak observed in all thermograms of the complexes at temperatures below the melting point of inclusion compounds, was probably due to the crystallization of amorphous inclusion compounds (22).

In the physical mixtures, the melting points of BPAA and methylated- and hydroxypropylated-β-Cyd are present but at lower temperatures. This shift is probably due to a weak interaction between host and guest (23).

Fig. 3 reports the powder X-Ray diffraction patterns of BPAA alone and of three complexes and the relative physical mixtures.

The diffraction patterns of the physical mixtures are the superposition of the patterns of each component, while those of the inclusion compounds show peaks different from those of the physical mixtures, indicating that a new solid phase exists.

The diffraction pattern of HP-ß-Cyd-BPAA complex is completely diffused, similar to that of HP-ß-Cyd. However the peaks of the BPAA molecule at 3.8, 18.8, 19.3 and 20.4°, present in the physical mixture, have disappeared indicating that BPAA interacts with HP-ß-Cyd and is transformed in an amorphous compound.

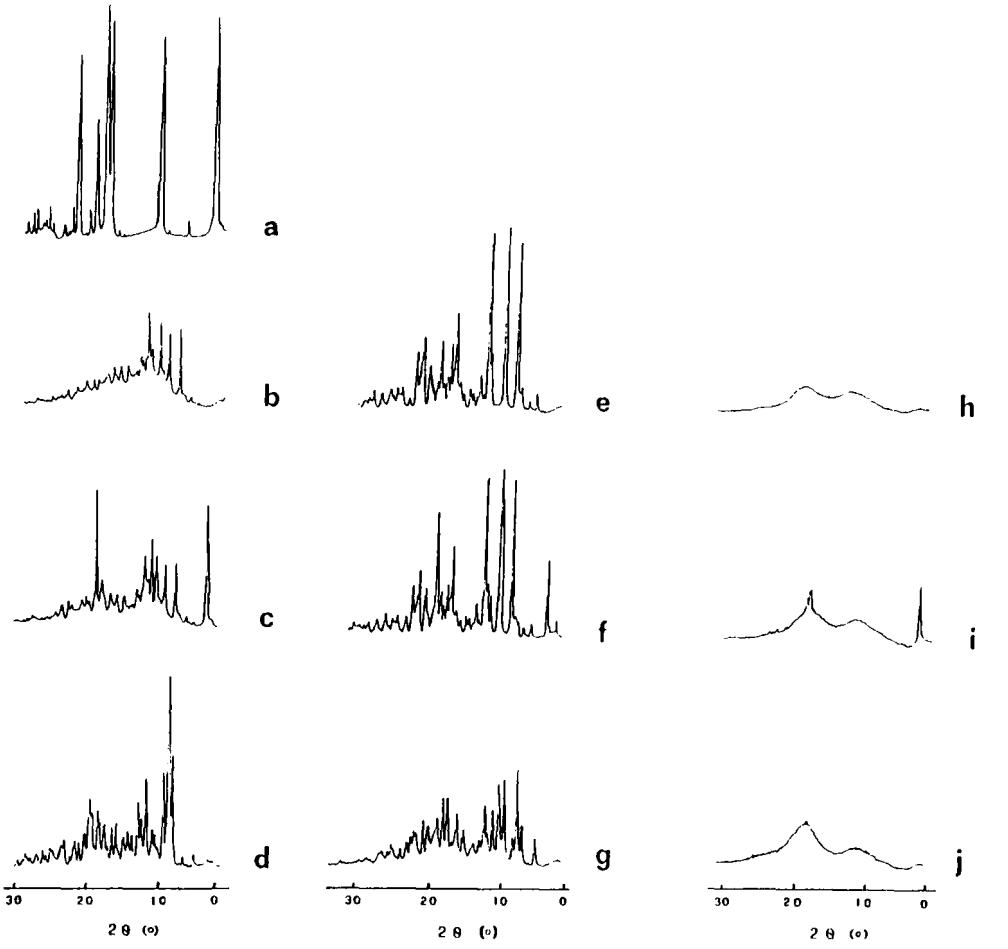


FIGURE 3

X-Ray diffraction patterns of β-Cyd derivatives-BPAA systems. a) BPAA; b)TM-β-Cyd; c) TM-β-Cyd-BPAA physical mixture; d) TM-β-Cyd-BPAA complex; e) DM-β-Cyd; f) DM-β-Cyd-BPAA physical mixture; g) DM-β-Cyd-BPAA complex; h) HP-β-Cyd-BPAA; i) HP-β-Cyd-BPAA physical mixture ; j) HP-β-Cyd-BPAA complex.

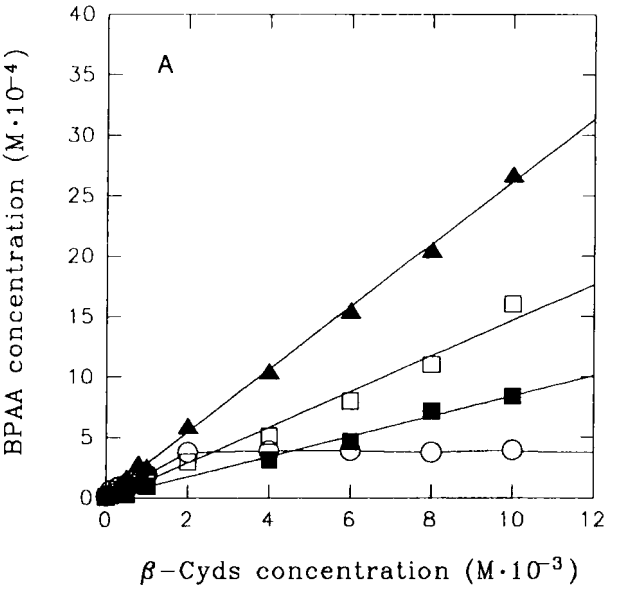


FIGURE 4 A

Phase solubility diagrams for BPAA with various β-Cyds in pH 1.1 buffer solution and at 25 ± 0.5°C. ○ = β-Cyd-BPAA; ■ = TM-β-Cyd-BPAA; □ = HP-β-Cyd-BPAA; ▲ = DM-β-Cyd-BPAA.

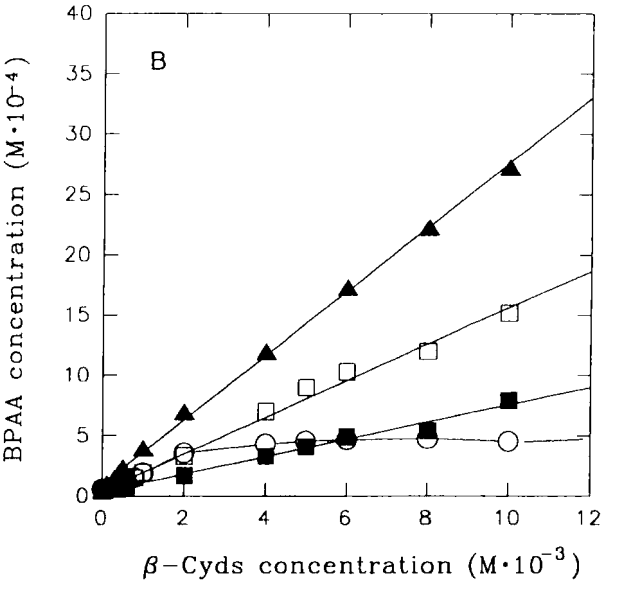


FIGURE 4 B

Phase solubility diagrams for BPAA with various β-Cyds in pH 1.1 buffer solution and at 37 ± 0.5°C. Symbols as in fig. 4 A.

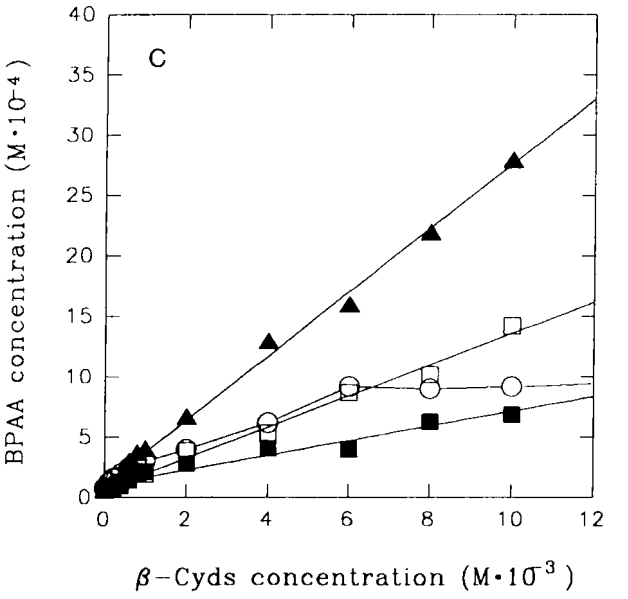


FIGURE 4 C

Phase solubility diagrams for BPAA with various β-Cyds in pH 1.1 buffer solution and at 45 ± 0.5°C. Symbols as in fig. 4 A.

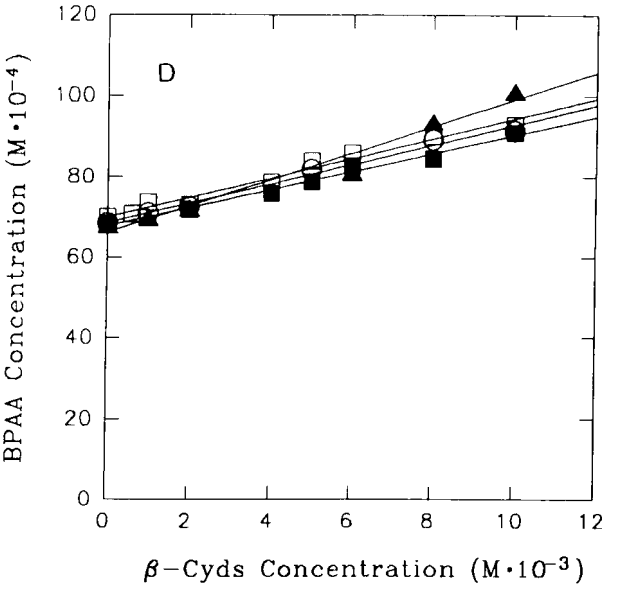


FIGURE 4 D

Phase solubility diagrams for BPAA with various β-Cyds in pH 7.4 buffer solution and at 37 ± 0.5°C. Symbols as in fig. 4 A.

β-Cyds-BPAA complexes in solution were characterized using solubility studies, UV and CD spectroscopies and NMR.

Fig. 4 A reports the effects of ß-Cyds on the solubility of BPAA at pH 1.1 and at 25 ± 0.5°C.

Linear plots were obtained in the case of TM-, DM- and HP-β-Cyd, indicating a first-order dependency on concentration of β-Cyd derivatives for the host-guest interactions. The resulting solubility curve can be classified as an AL type (17), and indicates that very soluble complexes are formed and no precipitation occurs at high concentrations of β-Cyd derivatives.

The intercept value observed for all plots represents the concentration of free drug in solution and the slope represents the complexating ability of the β-Cyds.

The solubility isotherm for β-Cyd is a Bs type, as reported in our previous study in water at 37 °C (11). The plateau observed in this plot indicates that a complex of limited solubility is formed at high β-Cyd concentration. From data in the plateau region, a 1:1 stoichiometry was determined for this complex in the solid phase.

The slope value of isotherm of β-Cyd- and HP-β-Cyd-BPAA systems is similar, indicating the similar complexating ability for BPAA (see table 1 for Kc values).

The solubility isotherms at 37 and 45 ± 0.5°C showed an enhancement of intercept value for all complexes, while the slope values are similar to those obtained at 25°C (fig. 4 B,C). The plateau of solubility curve of β-Cyd-BPAA system at 45°C occurs at higher β-Cyd concentration than that at 25°C. This behaviour could be due to the positive effect of the temperature on the complex solubility.

The solubility studies were performed also at pH 4.0 and 7.4. They showed a similar trend like that of pH 1.1 (plots not reported). The intercept values of all isotherms are higher, and particularly at pH 7.4 owing to ionization of BPAA at this pH value (pKa = 3.9). Althougth BPAA solubility at pH 7.4 increases with β-Cyds concentration, the complexating ability of all β-Cyds for a more hydrated BPAA is similar and significantly reduced (a decrease of slope values was observed).

The trend of solubility curve of β-Cyd-BPAA system at pH 7.4 and at the different temperatures (25, 37 and 45 °C) is different from that at pH 1.1 and 4.0. In fact, the solubility curve type changes in A_L (β-Cyd concentration range 0÷0.012 M) (fig.4 D). This effect is probably due to the decreased complexating ability of β-Cyd, which achieves a lesser β-Cyd-BPAA complex concentration in solution than that obtained at a pH value of 1.1. Therefore, the saturation solubility of complex is reached at higher β-Cyd concentration and a plateau is not observed.

In table 1 the Kc values of all complexes are shown. A decrease of Kc values at the increase of pH values was observed.

The Kc value of DM-β-Cyd-BPAA complex is greater than that of the others, probably because of the presence of methyl groups that expand the hydrophobic region of the DM-β-Cyd cavity and hence increase the affinity for BPAA.

As well as the hydrophobic interaction, steric hindrance also plays a role in the host-guest interaction; it is in fact interesting to note that although the TM-β-Cyd cavity has greater hydrophobicity compared to other β-Cyds, the Kc value of the TM-β-Cyd-BPAA complex is smaller, suggesting that the 3-O-methyl group reduces the interaction between BPAA and the host molecule.

The greater attraction of β-Cyds for BPAA in a more lipophylic form is evident from the correlation between log Kc of all complexes and the log PC of the drug determined at different pH values at 37 ± 0.5°C (fig. 5). The Kc values found are directly proportional to PC

TABLE 1

Physico-Chemical Parameters of BPAA and its Inclusion Complexes Obtained at Various Temperatures (°C) and pH Values.

	BPAA		**STABILITY CONSTANT OF COMPLEXES (mol/L)**											
	solubility (mol/L)	log PC	β-Cyd-BPAA complex			TM-β-Cyd-BPAA complex			DM-β-Cyd-BPAA complex			HP-β-Cyd-BPAA complex		
pH	37°	37°	25°	37°	45°	25°	37°	45°	25°	37°	45°	25°	37°	45°
1.1	$0.46 \cdot 10^{-4}$	4.45	7050	4519	2534	4120	1797	610	19800	7394	6303	6332	4166	2254
4.0	$0.65 \cdot 10^{-4}$	4.04	3079	1612	1290	840	731	685	5121	4067	3196	1851	1339	1180
7.4	$68.08 \cdot 10^{-4}$	3.62	192	60	43	160	48	35	201	85	60	175	51	41

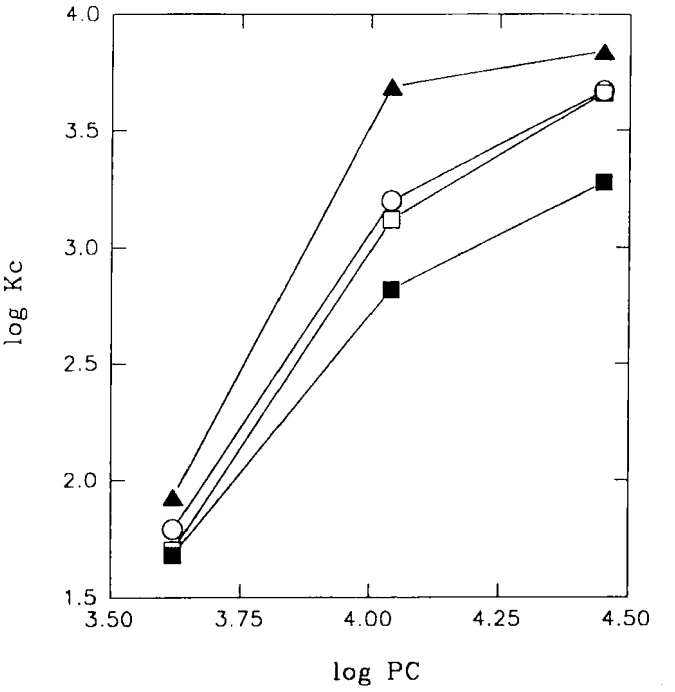


FIGURE 5

Relationship between stability constants of β-Cyds complexes and partition coefficients of BPAA. ○ = β-Cyd-BPAA; ■ = TM-β-Cyd-BPAA; □ = HP-β-Cyd-BPAA; ▲= DM-β-Cyd-BPAA.

values; in particular, a double trend of the plots was observed: Kc variations are in fact marked from PC = 3.62 to PC = 4.04 corresponding to BPAA pKa, they appear less evident above this value.

Table 2 reports the thermodynamic parameters of complexes at various pH values.

At pH 1.1 the enthalpic variations of all complexes are negative, indicating that complexation is always an exothermal process. In the formation of the inclusion complex, the energetic level of the system can be evaluated as the sum of the free-energy changes of several processes (24): 1) release of water molecules included in β-Cyds cavity; 2) desolvation of drug; 3) interaction of drug with β-Cyds cavity. Assuming that the energetic contribution of BPAA desolvation is the same for all complexes, the energetic level of the system is strictly dependent on the water release from β-Cyds cavity. The enthalpic value determined for TM-β-Cyd-BPAA complex is lower than those for β-Cyd-, HP-β-Cyd- and DM-β-Cyd-BPAA complexes. TM-β-Cyd probably has weaker interaction with water molecules due to the absence of free hydroxyl groups and therefore the splitting of this water requires less energy. Furthermore, the higher values of enthalpy for the β-Cyd-, HP-β-Cyd- and DM-β-Cyd-BPAA complexes are in agreement with the entropic values determined. The less negative values of ΔS found for β-

TABLE 2

Thermodynamic Parameters for Complexation of BPAA with β-Cyclodextrin, Methylated β-Cyclodextrins and Hydroxipropyl-β-Cyclodextrin

	BPAA-β-Cyd complex			BPAA-TM-β-Cyd complex			BPAA-DM-β-Cyd complex			BPAA-HP-β-Cyd complex		
pH	ΔH	ΔS	ΔG	ΔH	ΔS	ΔG	ΔH	ΔS	ΔG	ΔH	ΔS	ΔG
1.1	-9.4	-13.7	-5.3	-16.3	-37.9	-4.9	-11.2	-17.8	-5.9	-9.6	-14.6	-5.2
4.0	-8.3	-11.8	-4.8	-2.0	6.8	-4.0	-4.6	1.9	-5.1	-4.2	0.9	-4.5

ΔH and ΔG are in Kcal/mol and ΔS is in cal/°K/mol

Cyd-, DM-β-Cyd- and HP-β-Cyd-BPAA complexes with respect to the TM-β-Cyd-BPAA complex are due to less disorder of the ß-Cyd-, DM-ß-Cyd- and HP-ß-Cyd-water systems compared to TM-β-Cyd-water. These values probably reflect a more conformational rigidity of the cyclodextrin ring induced by the complexation with BPAA (e.g. formation of stable hydrogen bonds with BPAA, greater rigidity of β-Cyds ring following the complexation, etc.), as well as a decrease in degrees of rotational and translational freedom of the included drug.

Nevertheless, at pH 4.0 an increase in both the thermodynamic parameters was observed. Considering that at this pH the drug is partially ionized, the energetic gain due to the inclusion complexation is reduced. This would explain the observed ΔH increase. The ΔS increase is probably due to a reduction in hydrogen bonds between host and guest. Hence, the total ΔG substantially reflects the trend in ΔH.

The interaction between BPAA and β-Cyds was further examined by UV and CD spectroscopy.

Free BPAA has no CD band and β-Cyds have CD bands only at wavelengths below 220 nm. The CD band observed in fig. 6A in the wavelength region of the drug chromophore (254 nm), is, therefore induced by the asymmetric β-Cyds cavity that perturbs the electronic transition of the BPAA. The sign of the induced CD band depends on the spatial relationship between the asymmetric center and the perturbed chromophore (25). Thus the positive sign of CD bands for all complexes indicates that the biphenyl moiety of BPAA is similarly orientated in the cavity in all β-Cyds (26). The magnitude of the optical activity seems to depend on the rigidity of the inclusion compounds, consequently the smaller CD band of TM-β-Cyd-BPAA complex, indicates that the 3-O-methyl group prevented a strong interaction with BPAA. These results are in agreement with Kc values obtained in the solubility studies.

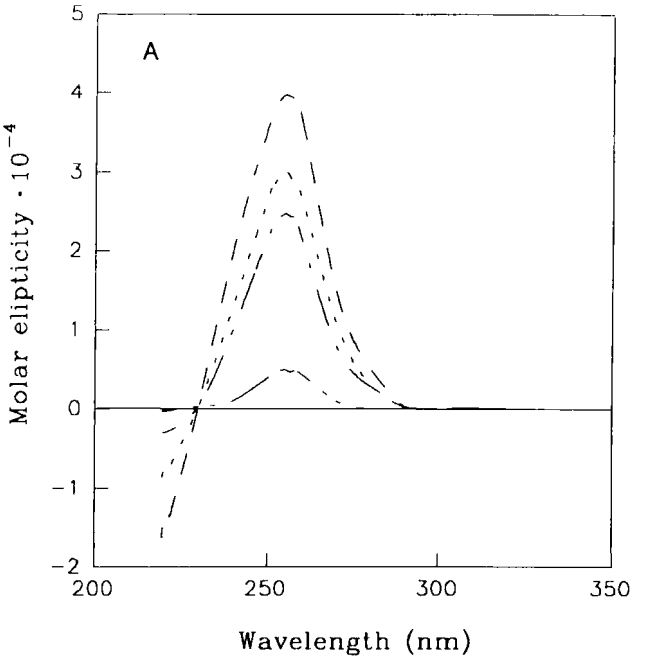


FIGURE 6 A

Circular Dichroism absorption spectra of BPAA in the presence of various β-Cyds in pH 1.1 buffer solution and at 37 ± 0.5°C. —— BPAA; - - - BPAA + β-Cyd; - —— - BPAA + TM-β-Cyd; — - — BPAA + HP-β-Cyd; —— —BPAA + DM-β-Cyd.

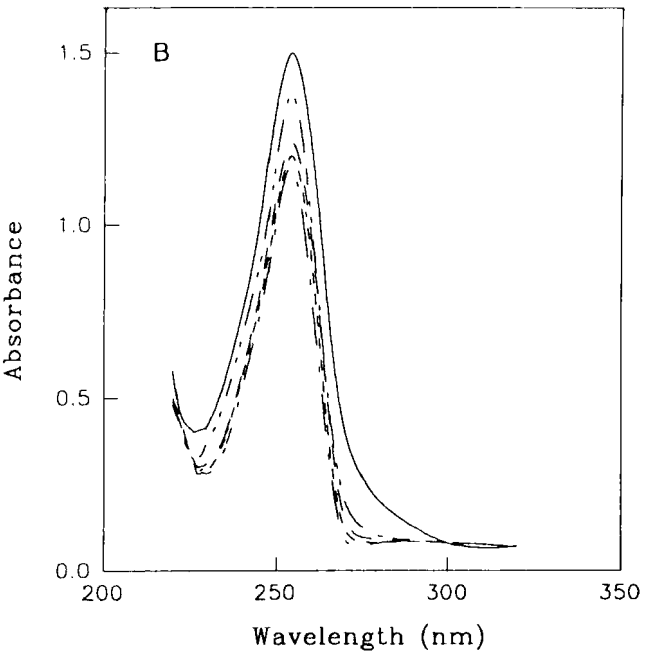
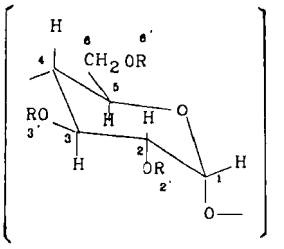


FIGURE 6 B

UV absorption spectra of BPAA in the presence of various β-Cyds in pH 1.1 buffer solution and at 37 ± 0.5°C. For courve attribution, see fig. 6A.

TABLE 3

Methylated β-Cyclodextrins ^{13}C Chemical Shifts and their Displacements in complexes with BPAA



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R = H, CH_3

	DM-β-Cyd			TM-β-Cyd		
C	without	with	$\Delta\delta^a$	without	with	$\Delta\delta^a$
1	99.266	99.412	0.146	96.700	97.612	0.912
2	80.765	80.773	0.008	79.648	80.625	1.010
3	72.099	72.067	-0.032	76.724	78.658	1.934
4	81.741	81.901	0.160	80.438	80.094	-0.344
5	69.162	69.415	0.253	69.990	69.753	-0.237
6	70.087	69.774	-0.313	70.360	70.258	-0.102
2'	57.554	57.835	0.281	57.675	57.411	-0.264
3'				59.349	60.128	0.779
6'	59.010	59.004	-0.006	57.932	57.888	-0.044

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

In fig. 6 B we report the UV spectrum of BPAA alone and in the presence of different β-Cyds. No batochromic shift was observed for BPAA in the presence of β-Cyds, but the intensity of the absorption maximum is slightly decreased as a result of a partial shielding of chromophore electrons within the β-Cyds cavity.

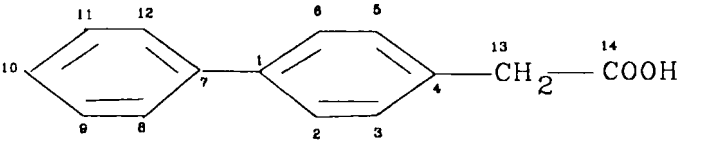
Nuclear Magnetic Resonance Studies

Experimental evidence of the inclusion, was provided by NMR studies. In particular, this technique was employed to gain further insight into the inclusion mode.

^1H-NMR spectra of TM-β-Cyd-BPAA and DM-β-Cyd-BPAA complexes, performed in D_2O, showed a change in chemical shift for the protons located in the hydrophobic cavity (H_3

TABLE 4

^{13}C Chemical Shifts of BPAA in Presence and Absence of Methylated β-Cyclodextrins



C	BPAA alone	with DM-β-Cyd	Δδ[a]	with TM-β-Cyd	Δδ[a]
1	137.703	137.210	-0.493	137.703	0
2,6b	126.151	125.431	-0.220	126.009	-0.142
3,5b	125.967	125.436	-0.531	126.122	0.155
4	135.910	136.951	1.041	136.863	0.953
7	139.505	139.955	0.450	140.246	0.741
8,12	128.197	128.265	0.068	128.195	0.020
9,11	129.090	129.224	0.134	129.216	0.130
10	126.574	127.022	0.448	126.747	0.173
13	43.652	43.881	0.231	43.915	0.263
14	180.090	178.771	-1.319	178.757	-1.333

a: Δδ = δcomplex - δfree

b: alternative assignments are possible

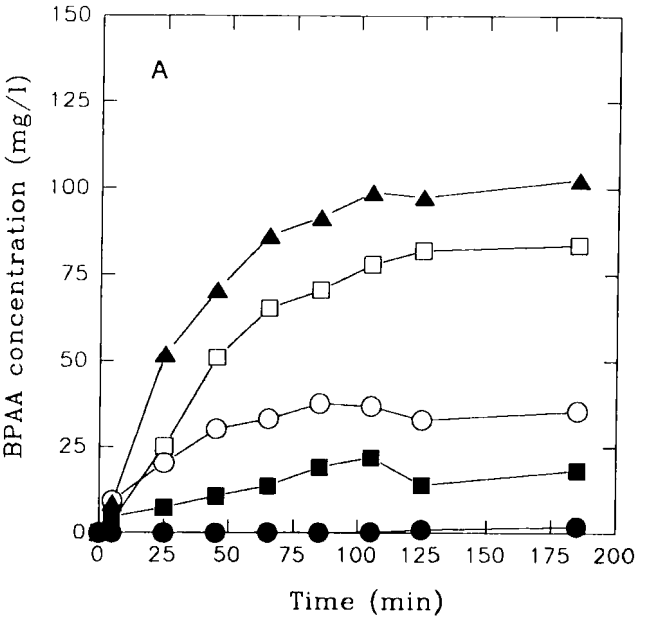


FIGURE 7 A

Dissolution profiles of BPAA complexes with β-Cyd and β-Cyd derivatives in buffer solutions at pH 1.1 at 37 ± 0.5°C. ● = BPAA; ○ = β-Cyd-BPAA; ■ = TM-β-Cyd-BPAA; □ = HP-β-Cyd-BPAA; ▲ = DM-β-Cyd-BPAA.

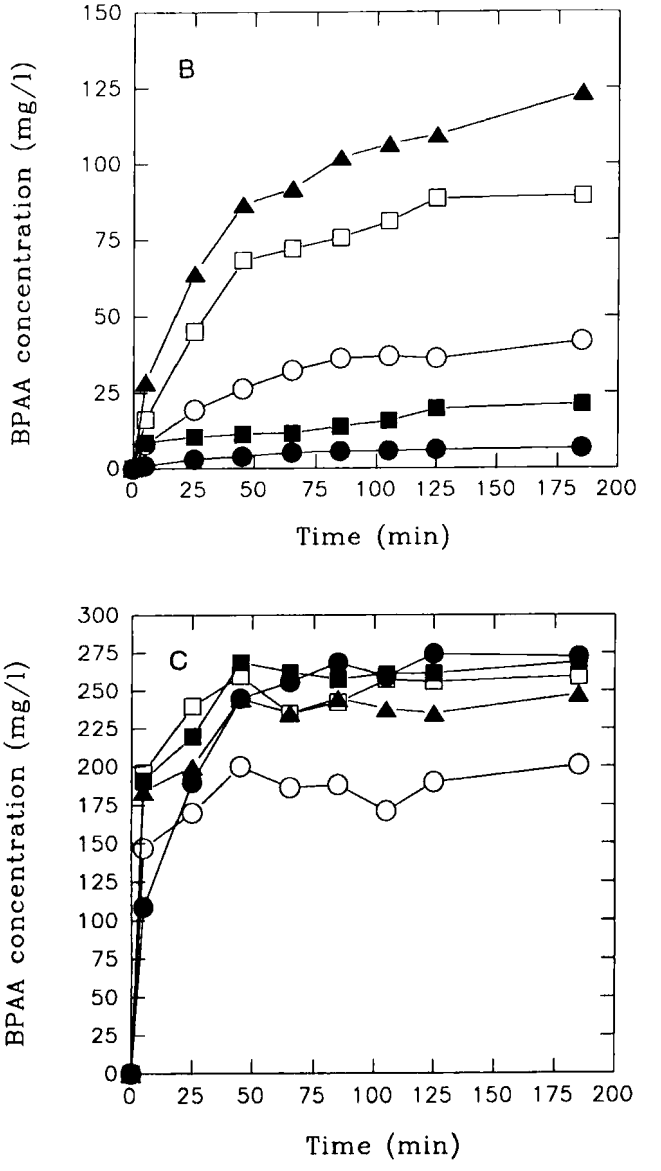


FIGURE 7 B,C

Dissolution profiles of BPAA complexes with β-Cyd and β-Cyd derivatives in buffer solutions at pH 4.0 (B) and 7.4 (C) at 37 ± 0.5°C. Symbols as in fig. 7 A

and H_5) compared to TM- and DM-β-Cyd alone, but it is not possible to clearly determine the shift of protons because they are overlapped by methoxyl proton signals. Protons located on the outside show very small or no shifts. Moreover, BPAA aromatic protons, present as a broad signal, are markedly deshielding in the complexes compared to the drug alone. These results verify an interaction between BPAA and methylated-β-Cyds.

A determination of stoichiometry of complexes cannot be made in aqueous media because of the low signal intensity. Therefore, in order to derive the molecular ratio, NMR spectra of complexes were performed in $CDCl_3$, where BPAA and methylated β-Cyds exhibit high solubility. Although a splitting of drug molecule by the solvent was observed, it is nevertheless possible to determine the stoichiometry in the original complexes (1:1 for the two complexes) from these spectra.

More evidence of complex formation was obtained from ^{13}C-NMR.

Table 3 summarizes the effects of BPAA on the ^{13}C-chemical shifts of methylated-β-Cyds.

It is evident that formation of the complex results in a notable change in carbon signals, particularly for TM-β-Cyd. The chemical shifts of C1 and C4 reflect a macrocyclic conformational change associated with the complexation (27,28). The marked changes in these carbons in TM-β-Cyd suggest that this macrocycle is significantly perturbed by BPAAcompared to DM-β-Cyd, probably due to the more distorted ring of the former (29). The other methylated β-Cyd carbon resonances reflect anisotropic ring current and steric compression interaction with the substrate (27). The C2 and C3 carbons in TM-β-Cyd are more deshielding than in DM-β-Cyd, probably as a consequence of the distorted structure of this Cyd ring. The shift of C6 carbon in DM-β-Cyd is more significant than in TM-β-Cyd, probably because in this case BPAA biphenyl moiety deeply penetrates into the cavity, while in the TM-β-Cyd, only the first phenyl group is introduced (28).

Table 4 reports chemical shifts of BPAA in the presence of two methylated-β-Cyds.

All carbon signals of the BPAA molecule were influenced by the addition of methylated-β-Cyds as a result of the hydrophobic interaction of host and guest molecules. The shielding of C14 does not indicate a direct interaction of the carboxilated group with the β -Cyd derivatives cavity, since it has been reported (30) that a much higher shift (~ 3ppm) is observed when the carboxilated group is transferred from D_2O solution into the less polar β-Cyd cavity.

The ^{13}C-NMR results obtained are in agreement with the data reported in the literature for analogous molecules (31).

Dissolution studies

Fig 7 A-C reports the dissolution profiles of BPAA and its complexes in buffer solutions at various pH values (1.1, 4.0 and 7.4) at 37 ± 0.5°C.

The pH of the dissolution medium has a clear effect on the dissolution profile. It is evident that all complexes dissolved more rapidly than BPAA itself at pH 1.1 and 4.0. This enhanced dissolution rate of BPAA may be due to the increase in solubility and wettability along with the decrease in cristallinity caused by the complexation (32). In the case of DM-β-Cyd-BPAA complex, which has a higher stability constant, the drug concentration following dissolution exceeded its normal solubility by as much as 55-fold. This supersaturation state was quite stable, and precipitation of the drug was not observed. The dissolution rate of TM-β -Cyd-BPAA complex is less than the others, probably because its low stability constant results in the dissociation of the complex after dissolution.

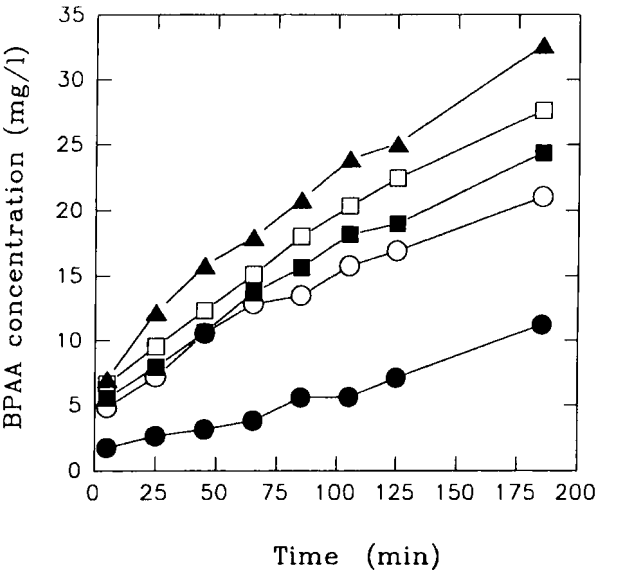


FIGURE 8

Diffusion profiles of BPAA and its β-Cyds inclusion complexes across an artificial lipid membrane in pH 1.1 buffer solution at 37° ± 0.5 °C. Symbols as in fig. 7 A.

TABLE 5

Kd Values of BPAA and BPAA-β-Cyds Complexes

COMPOUNDS	Kd (cm/min)
BPAA	$0.32 \cdot 10^{-2}$
β-Cyd-BPAA	$0.93 \cdot 10^{-2}$
TM-β-Cyd-BPAA	$1.21 \cdot 10^{-2}$
DM-β-Cyd-BPAA	$1.62 \cdot 10^{-2}$
HP-β-Cyd-BPAA	$1.54 \cdot 10^{-2}$

With increasing pH, the amount of free and included BPAA in solution increases, and at pH 7.4 the dissolution profiles of BPAA alone and complexed with β-Cyd derivatives were comparable. This trend is probably due to the very low Kc values of all the complexes at this pH, the BPAA being almost completely free and in a hydrated form. Under these conditions, the dissolution rate depends only on the solvation of BPAA.

Simulated absorption study

Fig. 8 shows the diffusion profile of BPAA across a lipid membrane at 37 ± 0.5°C, in the absence and presence of different β-Cyds.

It is evident that the diffusion process is faster for all complexes than for free BPAA, particularly for the DM-ß-Cyd-BPAA complex. The diffusion rates (Kd) of all complexes are given in table 5.

The diffusion process depends on dissolution rate, but also on the Kc values of complexes, because only BPAA in a free form can cross the lipid membrane. It was observed that TM-β-Cyd-BPAA complex diffused more rapidly than β-Cyd-BPAA complex, although its dissolution rate is lower. This fact is probably due to the low Kc value of the complex that compensates for the low dissolution rate.

CONCLUSIONS

The results obtained demonstrate that the aqueous solubility and dissolution rate of BPAA significantly increase when complexed with different β-Cyds, particularly with DM-β-Cyd. The ability of β-Cyds to form the complex with BPAA is inhanced at pH 1.1, in fact stability constants show a marked decrease at pH 7.4, demonstrating their reduced affinity to BPAA in its ionized form.

The diffusion rate of BPAA, across an artificial lipid membrane, determined at a pH 1.1 donor phase, increases as follows: BPAA<β-Cyd-BPAA<TM-β-Cyd-BPAA<HP-β-Cyd-BPAA<DM-β-Cyd-BPAA. From these results it appears that DM-β-Cyd is a better carrier for BPAA, because of its high water solubility.

"In vivo" studies of the complexes described are in course to evaluate the effect of β-Cyds carriers on the bioavailability and pharmacological activity of the drug.

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