International Journal of Pharmaceutics, 77 (1991) 247–254

IJP 02581

Inclusion complexes of bropirimine with β-cyclodextrin in solution and in solid state

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(Received 6 March 1991)
(Modified version received 4 June 1991)
(Accepted 2 July 1991)

Key words: Bropirimine; β-Cyclodextrin; Complexation; Ultrafiltration; Stability constant

Summary

Bropirimine was found to form an inclusion complex with β-cyclodextrin in aqueous solution and in the solid phase. Phase-solubility and UV spectroscopic studies revealed the formation of a 1:1 complex. The usefulness of the ultrafiltration technique for studying this interaction was proved and the stability constant of the complex was calculated using simple equations. Chemical-shift changes observed in the ^{1}H-NMR spectra of both the host and guest molecules clearly indicated complex formation and confirmed the penetration of part of bropirimine molecule into the β-cyclodextrin cavity. The formation of the complex in the solid state was confirmed by differential scanning calorimetry, CP-MAS ^{13}C-NMR, and infrared spectroscopy.

Introduction

Bropirimine (ABPP, 2-amino-5-bromo-6-phenyl-pyrimidin-4(3H)-one) is an antiviral, interferon inducer and anti-cancerogenic agent (Hamilton et al., 1980). It has a very poor aqueous solubility (Alpar et al., 1986), a property that decreases its gastro-intestinal absorption and impairs its bioavailability. This problem represents a limiting factor against formulation and clinical trials of this drug. Solid dispersion (Irwin and Iqbal, 1988) and cosolvency (Alpar et al., 1986) were proposed in order to solve this problem, however, these procedures suffer several disadvantages in practical use.

The rapidly growing number of papers and patents on cyclodextrins and their actual pharmaceutical uses motivated the idea of applying this approach. The main purpose of this study is to prepare and characterize an ABPP-β-cyclodextrin inclusion complex. The formation of such a complex was confirmed by a variety of techniques such as phase-solubility, ultrafiltration, UV spectroscopy, ^{1}H- and ^{13}C-NMR, differential scanning calorimetry (DSC) and infrared spectrophotometry (FTIR).

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Materials and Methods

Materials

Bropirimine was kindly supplied by Upjohn Co., U.S.A., β-cyclodextrin (BCD) was obtained from Roquette, Lestrem, France. Both were used as received. All other chemicals were of analytical reagent grade. For ultrafiltration, Diaflo YC05 membranes (43 mm diameter, lot no. Al 02519A; Amicon Corp., MA, U.S.A.) were used. Deionized, double-distilled water was used throughout the studies.

Methods

Solubility

Excess amounts of ABPP (20 mg) were equilibrated (7 days) by shaking with 10 ml aqueous clear solution of BCD (1.5×10^{-3}–1.5×10^{-2} M) in sealed conical flasks at constant temperature. Aliquots were pipetted through cotton filters and their drug contents were spectrophotometrically determined at 233 nm (Uvikon 810P, Switzerland) after appropriate dilution with water. Four temperatures were tested (25, 30, 37 and 45 °C), and each experiment was performed at least twice.

Ultrafiltration

Aqueous solutions (50 ml) of ABPP either alone or in the presence of different concentrations of BCD were filled into a stirred filtration cell (Amicon, model 8050). The solution was forced (pressure of 55 p.s.i. from nitrogen compressor) through the ultrafiltration membrane (MW cut-off 500) and collected in 3-ml fractions in succession. After collecting nine fractions, the pressure was released and the residual content of the cell was also collected. Fractions were analysed for ABPP spectrophotometrically at 306 nm. Each experiment was repeated at least three times.

UV spectrophotometry

The ABPP solution (3×10^{-4} M, 2 ml) was spectrophotometrically titrated by adding portions (100 μl each) of BCD solution (0.01 M) in succession. The changes in absorbance of ABPP at 233 nm were recorded against a blank containing the same concentration of BCD. In order to obtain more precise data from this titration, the drug and BCD were dissolved in low ionic strength phosphate buffer ($I = 0.01$, pH 7.4).

It should be noted that the drug exhibited two maxima at 233 and 306 nm. Both were found to be suitable for spectrophotometric assay of the drug.

Preparation of the solid complex

Both ABPP and BCD (1 : 1 molar ratio) were dissolved in water with the help of 7–10 drops of ammonia solution. The solution was driven off either by using a vacuum oven at 50°C (coprecipitated product) or by freeze drying (freeze-dried product, Modulyo apparatus, Edwards Co., Italy). No residual ammonia (Nessler's reagent) was detected in the solid products.

^{1}H-NMR

ABPP, BCD or the ABPP-BCD complex were dissolved (5–7 mg/ml) in a mixture of deuterium oxide (D_2O) and ammonium hydroxide-d_3 (20% ND_3 in D_2O, Merck) at pH 9. A Bruker CXP 300 spectrometer (300 MHz) was employed for recording the spectra. Experimental conditions were as follows: 128 pulse, time domain 8 K, repetition time 1.8 s and temperature 23°C. The chemical shifts were referred to the signal of residual HOD in the solvent.

CP-MAS ^{13}C-NMR

The spectra were recorded with a Bruker CXP-300 spectrometer at 75 MHz. The cross-polarization was 1 ms while the repetition time and the ^{1}H 90° pulse were 4 s and 4.75 μs, respectively. The chemical shifts were measured with respect to tetramethylsilane (TMS), with benzene as secondary substitution reference (128 ppm): number of scans, 1000–3000; rotational speed, 3.4 kHz.

Thermal analysis

DSC scanning was performed with a Perkin Elmer DSC-4 apparatus.

Samples (2–5 mg) were encapsulated in aluminium pans and program heated at a rate of

4°C/min in a dynamic nitrogen environment from to 50–350°C. The instrument was calibrated with indium. Duplicate measurements were carried out on each sample.

Infrared spectroscopy (FTIR)

The FTIR spectra were recorded on a Bruker IFS 66 spectrometer connected with a Harrick diffuse-refractance (Drift). A minimum of 200 scans were signal averaged at a resolution of 4 cm^{-1}. Interference from water vapour bands was reduced by smoothing the spectra prior to plotting. The frequency scale was internally calibrated with a reference helium-neon laser to an accuracy of 0.2 cm^{-1}.

Results and Discussion

Solution phase

Fig. 1 shows the equilibrium phase solubility diagram obtained for the ABPP/BCD system in water at four temperatures. It was found that the solubility of ABPP increased linearly as a function of BCD concentration and showed the features of an AL type (Higuchi and Connors, 1965). The apparent stability constant, $K_{(1:1)}$, of the soluble complex at each temperature was calculated according to the equation of Higuchi and Connors (1965), Eqn 1, where S is the slope of the line and C_s is the drug solubility at this temperature.

$$K_{(1:1)} = \frac{S}{C_s(1-S)} \tag{1}$$

Additionally, the thermodynamic parameters of the complex obtained from the temperature dependence of the dissociation constant were calculated (Table 1). The negative enthalpy change reflects an exothermic interaction and suggests the involvement of dipoles and/or hydrogen bonds in the formation of the complex, while the negative entropy change implies that hydrophobic interactions are not predominant for the interaction of ABPP and BCD in solution. As a result of the negative enthalpy change, a decrease in the standard free energy is observed.

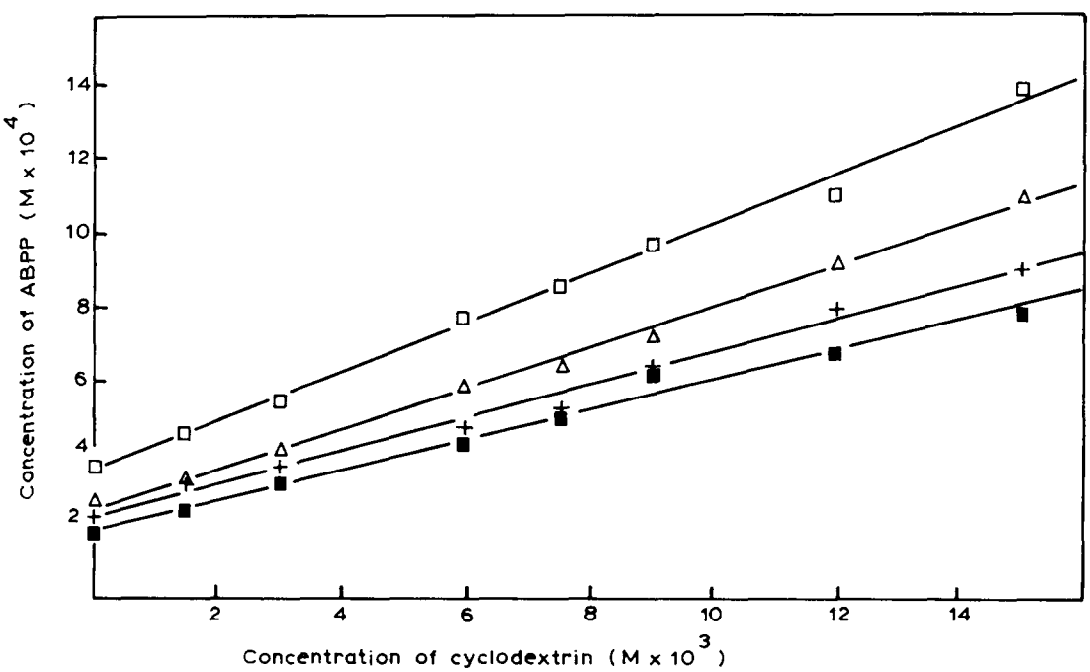


Fig. 1. Phase-solubility diagram of ABPP-BCD system in water at four temperatures: (■) 25, (+) 30, (△) 37 and (□) 45°C.

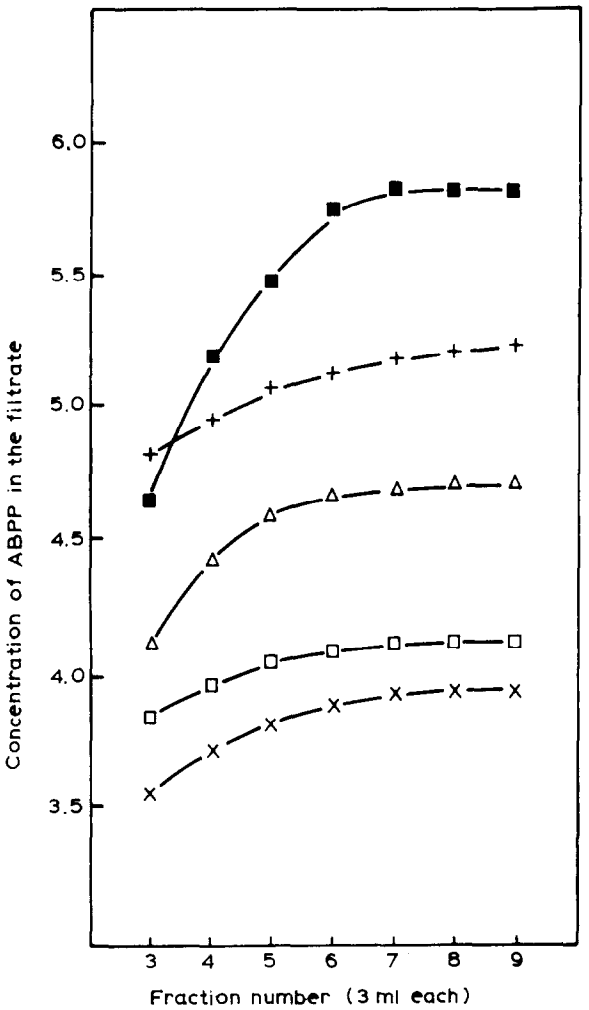


Fig. 2. Ultrafiltration of ABPP solutions (6×10^{-5} M) in the absence (■), and presence of 6×10^{-4} (+), 1.2×10^{-3} (△), 1.8×10^{-3} (□) and 2.4×10^{-3} (×) M BCD.

The ultrafiltration technique was successfully employed as a relatively fast and easy method for characterization of ABPP-BCD complex formation in solution. This technique was first applied by Jones and Parr (1983) for studying inclusion complexation. In our investigation, more detailed studies were performed and a simple equation for calculating the complex stability constant is appropriate. Among several membranes tested, the Diaflo YC05 was found to be the most suitable and effective membrane and could be considered as allowing ABPP to pass freely. A minor amount (≈ 3%) was retained upon filtering the drug alone (Fig. 2). This may be due to some structural features of the molecules to be filtered, e.g. size, shape, bond lengths and degree of hydration, which affect the retention by membranes of small molecular weight cut-off.

Fig. 2 also shows the ultrafiltration profiles of ABPP in the presence of different concentrations of BCD. During the early stages of the effluence the concentration of the drug is low, but the filtrate concentration reaches a constant level after 15 ml filtration. A distinct fall in the amount of ABPP filtered ($[ABPP]_f$, amount free) with increasing concentration of BCD contained in the

TABLE 2

Chemical shifts for BCD protons upon complexation with ABPP

BCD protons	β-Cyclodextrin alone	Bropirimine-β-cyclodextrin inclusion complex	
	Chemical shift (ppm)	Change in chemical shift in the presence of ABPP (ΔHz)	
		1:1 M	2:1 M
H-1	5.09	0.00	0.00
H-2	3.67	−0.88	−1.28
H-3	3.98	−6.96	−10.80
H-4	3.59	0.68	0.95
H-5	3.93	−1.50	−5.8

Negative signs indicate upfield displacement and vice versa. ΔHz is the difference in chemical shifts of BCD protons in the presence and absence of ABPP, referred to H-1.

TABLE 1

Apparent stability constants and derived thermodynamic parameters for the interaction between ABPP and BCD in water at 25°C

Stability constant $K_{(1:1)}$ (M^{-1})				ΔG° (cal mol^{-1})	ΔH° (cal mol^{-1})	ΔS° (cal mol^{-1} K^{-1})
25 °C	30 °C	37 °C	45 °C			
257	225.5	215.3	197	−910	−2251	−4.5

cell was observed. Undoubtedly, this indicates the formation of an ABPP-BCD complex having a higher combined molecular weight than the free drug molecule alone.

The stability constant of the ABPP-BCD complex could be calculated from the following equation,

$$K_{(1:1)} = \frac{[\text{ABPP-BCD}]}{[\text{BCD}][\text{ABPP}]_f} \quad (2)$$

where [ABPP-BCD] and $[ABPP]_f$ are the concentrations of the complexed and free drug, respectively. Assuming that the total concentration, $[ABPP]_t$ of the drug is given by the sum of the complex and the free drug, and BCD is present in excess, then Eqn 2 may be rewritten as

$$[\text{ABPP}]_f = \frac{[\text{ABPP}]_t}{K_{(1:1)}\text{BCD} + 1} \quad (3)$$

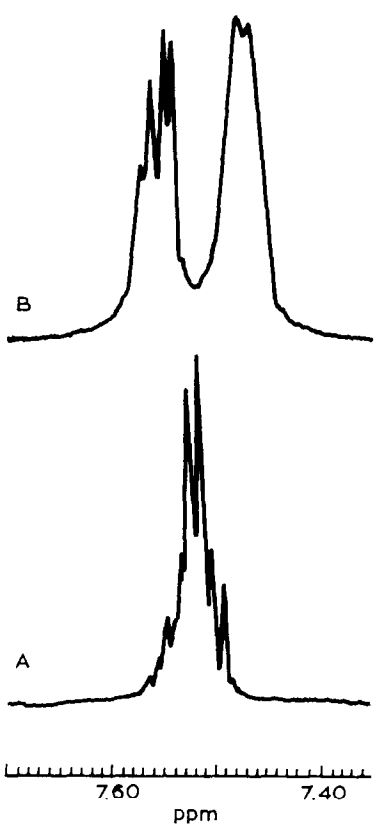


Fig. 3. ^{1}H-NMR for ABPP/BCD system. (A) ABPP alone; (B) ABPP/BCD complex (1 : 1 M).

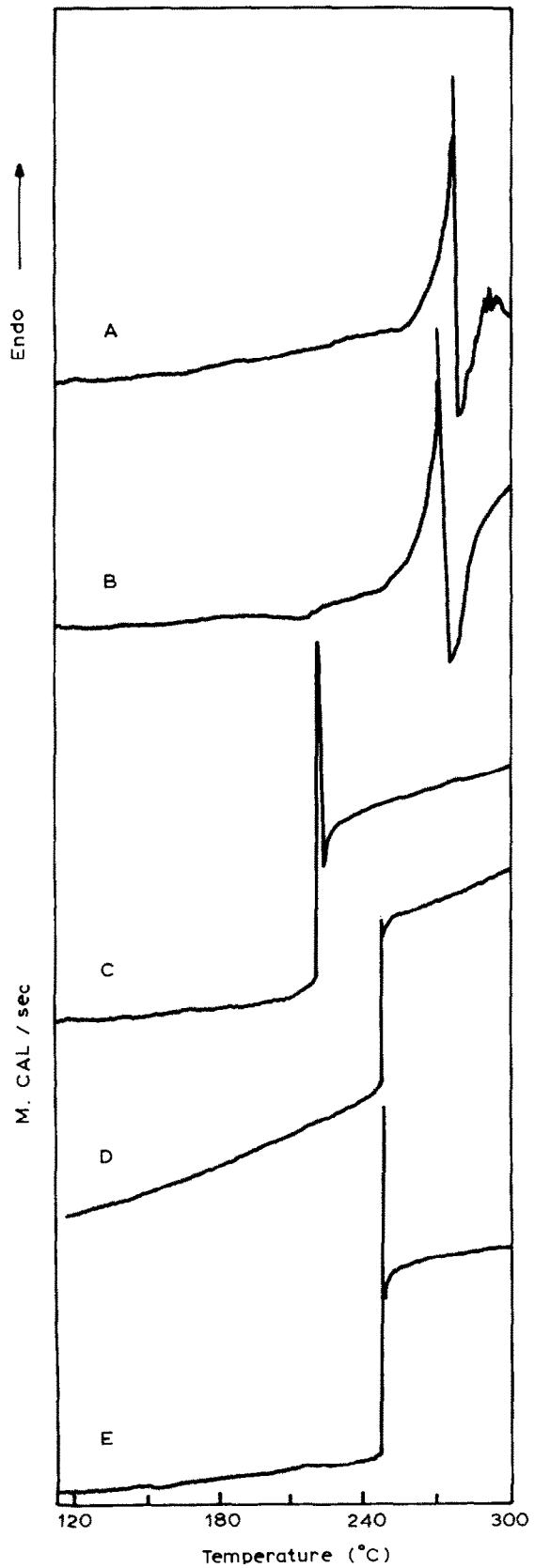
The value of $[ABPP]_f$ could be directly obtained from the mean value of the effluent concentrations after reaching equilibrium, i.e. the plateau region of each ultrafiltration profile. Since the $[ABPP]_t$ and BCD concentrations were arbitrarily chosen, the value of $K_{(1:1)}$ could readily be calculated. Accordingly, the value of $K_{(1:1)}$ for the ABPP-BCD complex was found to be $255 \pm 14.4\ M^{-1}$ (mean $\pm$ SD, for 5 determinations). This is in good agreement with the value obtained from the solubility method (Table 1, at 25°C) where the room temperature during ultrafiltration was 23 ± 2°C.

When solutions of ABPP were spectrophotometrically titrated with increasing amounts of BCD, a decrease in intensity of the maximum absorbance of the drug at 233 nm was observed. These changes may be due to perturbation of the electronic energy levels of the drug as a result of complex formation with BCD (Uekama et al., 1985).

According to the expression (Eqn 4) reported by Benesi and Hildebrand (1949):

$$\frac{1}{\Delta A} = \frac{1}{K_{(1:1)}[\text{ABPP}]_t \Delta\epsilon[\text{BCD}]} + \frac{1}{[\text{ABPP}]_t \Delta\epsilon} \quad (4)$$

where ΔA is the difference in absorbance, $\Delta\epsilon$ represents the difference in the molar absorptivities between free and complexed drug, $[ABPP]_t$ is the total ABPP concentration and [BCD] corresponds to the concentration of unbound BCD (which can be assumed to equal total BCD at high concentration). When $1/\Delta A$ was plotted vs $1/[BCD]$, a linear plot, having the least-squares equation: $1/\Delta A = 0.0434 \times 1/[BCD] + 12.8$ and a correlation coefficient of 0.982, was obtained. This indicates the formation of a 1 : 1 complex between ABPP and BCD. The value of the stability constant, $K_{(1:1)}$, calculated from the intercept-to-slope ratio, was found to be 295 M^{-1}. The presence of phosphate ions as buffer component may account for the somewhat higher value of $K_{(1:1)}$ than that calculated from the solubility or



ultrafiltration techniques. This may be attributed to change in the activity of the water due to hydration of the salt (Mochida et al., 1973).

Table 2 summarizes the effects of ABPP on the ^{1}H-NMR chemical shifts of BCD. It is evident that the H-3 and H-5 protons were shifted upfield whereas the H-1, H-2 and H-4 protons showed only small shifts. This effect became more pronounced with increasing molar concentration of ABPP relative to BCD. These results are similar to those first described by Thakkar and Demarco (1971) who attributed these changes to 'ring current' effects of the aromatic nucleus included in the cyclodextrin macrocycle. Moreover, the effects of BCD on the ^{1}H-NMR chemical shifts of ABPP were investigated (Fig. 3). It was observed that upon complexation, the ABPP signals were split into two groups, one shifted upfield and the other downfield. These data suggest that at least part of the ABPP molecule, most likely the phenyl moiety, penetrates into the BCD cavity.

Solid phase

The CP-MAS ^{13}C-NMR spectra of lyophilized BCD and of the ABPP-BCD complex prepared by the lyophilization method were compared (spectra not shown). No appreciable signals from the guest molecule were observed, probably because the amount of the guest molecules is low compared with that of the glucose residues of cyclodextrin. Nevertheless, the spectrum of the complex showed some broadening of the C-4 and C-6 signals of BCD at 62.5 and 104.8 ppm, respectively. These spectral changes might arise from a macrocyclic conformational change associated with the partial inclusion of the guest molecules. Similarly, Uekama et al. (1985) were able to suggest the modes of interaction of prednisolone with cyclodextrins using the same tech-

Fig. 4. DSC thermograms of ABPP/BCD system. (A) ABPP as received; (B) ABPP lyophilized; (C) ABPP/BCD physical mixture; (D) ABPP/BCD inclusion complex, lyophilized product; (E) ABPP/BCD inclusion complex, coprecipitated product.

nique. The signal assignments were made with reference to their article.

When pure ABPP as received or lyophilized was subjected to DSC analysis, it showed an endothermic peak at 273 °C which develops into an irregular exotherm (Fig. 4, traces A and B). During their detailed studies on the thermal stability of ABPP, Irwin and Iqbal (1989) stated that the thermal stability of the drug decreased upon admixture with several common excipients, including BCD, due to the presence of the labile $=C-Br$ bond in the molecule. A similar behaviour was observed during the present investigation where ABPP/BCD (physical mixture or inclusion complex) exhibited anomalous thermal events with onset temperatures of 225 and 250°C for the physical mixture and the complex, respectively (Fig. 4, traces C–E). The occurrence of such thermal events at a higher temperature in the case of the complex as compared with the physical mixture suggests the presence of some sort of inclusion of ABPP in the BCD cavity.

In fact, this could be explained by the protective effect of BCD against thermal degradation of the drug molecule, or by the change in average bond dissociation energies of the $=C-Br$ bond. We agree with Irwin and Iqbal (1988) who stated that such a thermal instability event should not cause any formulation or manufacturing problems, since it happens at temperatures above 200°C and is not due to direct chemical involvement of BCD in the reaction.

Further supporting evidence for the complex formation was obtained from the FTIR spectra in the 'fingerprint region' (1000–400 cm^{-1}) of the ABPP-BCD solid complex (1 : 1 M) in comparison with that of a 1 : 1 M physical mixture of the drug and BCD. As shown in Fig. 5, the frequency and intensity of the aromatic CH out-of-plane (800–650 cm^{-1}) bending modes of ABPP, especially that at 694 cm^{-1}, are significantly different in the complex. This suggests an interaction between the aromatic ring of the drug and the hydrophobic cavity of the BCD. Additionally, significant changes are also evident in the higher frequency region of the spectrum (not shown) where the C=O, NH_2 and NH stretching modes of ABPP occur. This also suggests that the non-aromatic

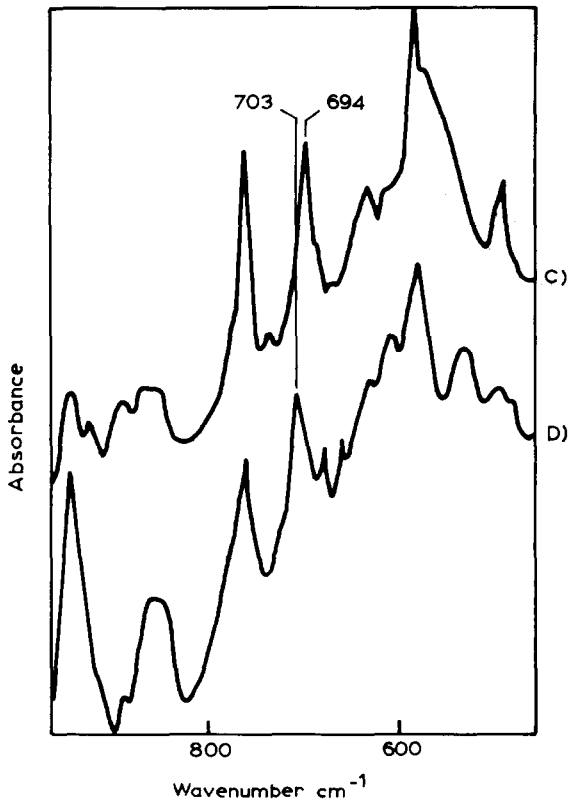


Fig. 5. FTIR spectra for ABPP-BCD system. (C) ABPP/BCD physical mixture (1 : 1 M); (D) ABPP/BCD inclusion complex (1 : 1 M).

moiety of the drug interacts with the cyclodextrin essentially through intermolecular hydrogen bonding, possibly with OH groups of the cyclodextrin rim. A more detailed IR study is in progress in order to verify this hypothesis.

It is worth mentioning that preliminary studies showed that BCD is more suitable for inclusion complexation of ABPP than α- or γ-CD, probably because of the appropriate size of its internal cavity.

In conclusion, bropirimine was found to form an inclusion complex with BCD. The solid complex could be prepared by either freeze-drying or coprecipitation techniques. The present findings provided some basis for formulation design of the drug and a means of improving its solubility. Other pharmaceutical properties of ABPP-BCD complex are currently under investigation.

Acknowledgements

This work was supported by a grant to S.M.A. from the Third World Academy of Science (TWAS; Trieste, Italy). The authors would like to acknowledge the technical assistance of M. Canetti in obtaining the DSC thermograms. The generous help of and fruitful discussions with Professor B. Casu (Institute of Chemistry and Biochemistry G. Ronzoni) are gratefully acknowledged.

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