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European Journal of Pharmaceutical Sciences 9 (2000) 381–386

EUROPEAN JOURNAL OF PHARMACEUTICAL SCIENCES

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Complexation of the interferon inducer, bropirimine, with hydroxypropyl-β-cyclodextrin

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Received 30 July 1999; received in revised form 20 October 1999; accepted 25 October 1999

Abstract

Bropirimine (ABPP) is an orally active immunomodulator that increases endogenous alpha-interferon and other cytokines used clinically against carcinoma in situ of the bladder. The oral absorption of ABPP is poor because its low solubility in water. The purpose of this study is to develop a technological procedure useful to increase the water solubility of ABPP. To this end, the interaction of ABPP with several cyclodextrin derivatives-α-, β-, γ- and hydroxypropyl-β-cyclodextrin with a degree of substitution 2.7 (HPβCD) was studied and the effect of the complexation process on the water solubility of the drug was evaluated. The best results were obtained with the hydroxypropyl derivative, HPβCD, that interacts in a 1:1 drug:cyclodextrin molar ratio. The inclusion complex ABPP–HPβCD was characterized in solution by nuclear magnetic resonance (^{1}H-NMR). The solid inclusion complex was obtained by freeze-drying and characterized by differential scanning calorimetry (DSC), X-ray diffractometry and mass spectrometry. The dissolution rate of ABPP from the HPβCD solid inclusion complex was increased compared to the powdered drug but not differences were found between the complex and a physical mixture with a similar molar ratio. The increase of the dissolution rate of the drug can be attributed to the breakdown in solution of the drug dimers in the presence of the cyclodextrin and to the complex formation.

Keywords: Bropirimine; Cyclodextrin complexation; Hydroxypropyl-β-cyclodextrin; Inclusion complex; Characterization; Solubility

1. Introduction

Bropirimine (2-amino-5-bromo-6-phenyl-4(3)-pyrimidinone, ABPP) (Fig. 1) is an orally active immunomodulator that increases endogenous alpha-interferon and other cytokines. It has been demonstrated that it has an activity against carcinoma in situ of the bladder (Rios et al., 1986) when taken orally at high doses. However, its low aqueous solubility — less than 0.1 mg/ml in aqueous solution pH 5–7.5 (Alpar et al., 1986) — determines a low oral bioavailability and doses as high as 3 g/day orally for three consecutive days each week up to one year, have been successfully used in this treatment (Sarosdy et al., 1996). It has been proved that the dissolution process is the rate-determining step for the ABPP absorption from the rat small intestine after dosing in suspension (Emori et al., 1995, 1996). The development of oral dosage forms which exhibit fast ABPP dissolution rate is a requisite to improve its oral bioavailability.

In recent years, the complexation with cyclodextrins is one of the most extended approaches used to modify some physicochemical properties such as aqueous solubility and,

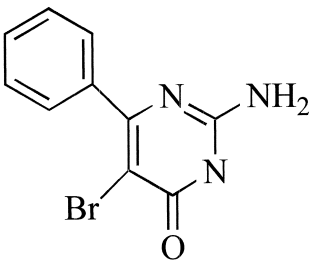

Fig. 1. Chemical structure of bropirimine (2-amino-5-bromo-6-phenyl-4(3)-pyrimidinone-ABPP).

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0928-0987/00/$ – see front matter
PII: S0928-0987(99)00081-0

some biopharmaceutical properties (Ahn et al., 1997; Becket et al., 1999; Miyake et al., 1999). Cyclodextrins are cyclic oligosaccharides consisting of 6, 7 or 8 α-1,4-linked glucopyranose units, and are usually referred to as α, β or γ-cyclodextrin, respectively. The cyclodextrin cavity exhibits a hydrophobic character, whereas the exterior of the molecule is hydrophilic. This particular structure allows various guest molecules to be included in the cavity to form what is called inclusion complexes. Many derivatives of these natural compounds have been synthesized to modify their physicochemical and biopharmaceutical properties. Hydroxypropyl derivatives, mainly of β-cyclodextrin, have been extensively used in pharmaceutical formulation owing to its high water solubility (>50% w/v) and low toxicity (Pitha et al., 1994; Carpenter et al., 1995; De Repentigny et al., 1998). They have shown their potential for parenteral use as well as being included in oral formulations because of the positive effect on the solubility and bioavailability of the drugs (Brewster et al., 1989, 1997; Järvinen et al., 1995).

Previous studies reported that the solubility of ABPP may be improved by the bile salts (Emori et al., 1995) or by its formulation in solid dispersions or inclusion compounds with β-cyclodextrin (Ahmed et al., 1991, 1993). However, the reported solubility change of ABPP by its complexation with β-cyclodextrin is limited because of the formation of poorly soluble complexes. Hydroxypropyl-β-cyclodextrin derivatives are particularly useful for the conversion of crystalline drugs to amorphous forms with an improved dissolution. Therefore, in the present study the complexation of ABPP with hydroxypropyl-β-cyclodextrin, has been investigated with the aim of achieving a greater dissolution rate of ABPP than for that observed when complexed with parent cyclodextrin. The formation of the complex has been assessed by phase-solubility diagrams, differential scanning calorimetry, X-ray diffractometry, mass diffractometry and ^{1}H nuclear magnetic resonance.

2. Material and methods

2.1. Materials

Bropirimine was given by Laboratorios Inibsa, S.A. (Spain). The cyclodextrins used in this study were: α-cyclodextrin (α-CD), γ-cyclodextrin (γ-CD) purchased from Cyclolab (Hungary) and Wacker-Biochem (Germany), respectively; β-cyclodextrin (β-CD) was donated by Roquette-Laisa España (Spain) and hydroxypropyl-β-cyclodextrin (average degree of substitution 2.7) (HPβCD) by Janssen Pharmaceutica N.V. (Belgium). Milli-Q water was used throughout all of the study and, all other chemicals were of analytical-reagent grade.

2.2. Methods

2.2.1. Solubility studies

Solubility studies were carried out according to Higuchi and Connors (1965). An excess amount of ABPP (20 mg) was added to 10 ml of cyclodextrin solution with different concentrations (β-cyclodextrin, 0–1.3% w/v and other cyclodextrins derivatives, 0–5% w/v). The suspensions were stirred in glass ampoules at 37°C and, shielded from light, until reaching equilibrium (about 7 days). Then, the content of each vial was filtered using 0.45 μm membrane filters (Millipore) and analyzed by HPLC to determine the drug concentration. Experiments were conducted in duplicate. Apparent 1:1 stability constants (K_c) of the ABPP–CDs complexes were calculated from the slope of the straight portion of the phase solubility diagrams (reporting drug concentration vs. CD concentration), using the equation:

$$K_c = \frac{\text{slope}}{S_0(1 - \text{slope})}$$

where S_0 is the solubility of the drug in water.

2.2.2. Physical mixture preparation

The physical mixture of ABPP and HPβCD in a 1:1 molar ratio was prepared by pulverizing and subsequent mixing in a Turbula T2C mixer (5 min at 30 rpm).

2.2.3. Preparation of solid inclusion complexes by ammonium hydroxide freeze-drying method

ABPP and HPβCD in 1:1 mol:mol ratio were dissolved in an aqueous 0.14% w/v solution of ammonium hydroxide. After dissolution was completed, the solution was filtered through a 0.45 μm membrane filter and frozen by immersion in liquid nitrogen. Freeze-drying was completed in 24 h in a Lyph-lock 6 equipment (Labconco). Nessler reagent in the freeze-dried product did not detect any ammonium ions.

2.2.4. ^{1}H-NMR studies

Nuclear magnetic resonance spectra of ABPP and HPβCD in different molar ratio were recorded on a Brücker AMX (300 MHz) spectrometer (Brücker Anal.) at 25°C. Samples were dissolved in 0.2 M deuterated sodium hydroxide. The internal reference was a peak due to small amounts of DHO and H_2O as impurities (assigned a value of $\delta = 4.6$ ppm).

2.2.5. Thermal analysis

Differential scanning calorimetry (DSC) was performed on a Shimadzu DSC-50 system with a DSC equipped with a computerized date station TA-5 WS/PC. The general conditions were: scanning rate 10°C/min, scanning temperature range 50–300°C.

2.2.6. X-ray diffractometry

X-ray power diffraction patterns were recorded on a Philips X-Ray diffractometer (PW 1710 BASED) using CuK_{α} radiation.

2.2.7. Mass diffractometry

FAB mass spectra were recorded on a Kratos MS-59 spectrometer.

2.2.8. Dissolution studies

In vitro dissolution studies of the pure drug, physical mixture and the inclusion complex were carried out using the USP 1 basket method, rotating at 50 rpm in 900 ml of simulated intestinal fluid without enzymes and maintained at 37°C. The samples, containing 150 mg of drug were placed in a hard shell colorless gelatin capsule. Simulated intestinal fluid without enzymes, pH 7.5±0.1, was prepared according to USP23Ed. with monobasic potassium phosphate and sodium hydroxide.

The drug concentration was measured by HPLC. The HPLC system consisted of an auto-injector Waters 600 Controlled (20 μl), a Waters 717 plus Autosampler and a Waters 996 Photodiode Array Detector, controlled by Waters Millennium PDA software. For the stationary phase, a reverse-phase column (Symmetry C-18, FX 5166, 150×3.9 mm) was used. The mobile phase was a mixture of methanol, water (acetic acid 0.2%) 48:52 v/v with a flow rate of 1 ml min^{-1} and, was deaerated on-line bubbling helium gas. Detection was performed at 233 nm. All samples were analyzed in triplicate.

Dissolution efficiencies after 180 min were calculated according to Khan (1975). The effects of drug formulation on the dissolution efficiency was investigated by one-way analysis of variance (ANOVA) with the Least Significant Difference (LDS) test for multiple comparisons. Differences were considered to be significant at a level of $\alpha < 0.05$.

3. Results and discussion

The solubility calculated for ABPP in water, pH 6.5, was 80 μg/ml which increases with the addition to the solution of all the cyclodextrins derivatives used. The solubility curves of ABPP in water are depicted in Fig. 2. According to the classification introduced by Higuchi and Connors (1965), all phase solubility diagrams can be classified as type A_L, i.e., the solubility of ABPP increased linearly with the increasing concentration of CDs. Table 1 shows the stability constants calculated for all cyclodextrins. The solubilizing effect of cyclodextrin derivatives is in the following order:

HPβCD > β-CD > γ-CD > α-CD

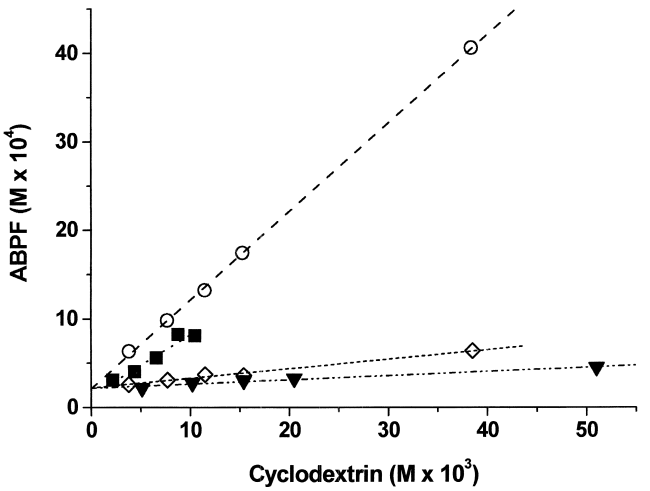

Fig. 2. Phase solubility diagrams for ABPP in the presence of α-CD (▼), β-CD (■), γ-CD (◇), HPβCD (○) in water at 37±0.5°C ($n=2$).

The enhancement in solubility at the highest cyclodextrin concentration was 16.95 fold for HPβCD (5% w/v), 3.37 fold for β-CD (1.2% w/v), 2.67 fold for γ-CD (5% w/v) and 1.88 fold for α-CD (5% w/v) system. Therefore, ABPP forms the most stable inclusion complexes with HPβCD and, because the solubilizing effect obtained with this derivative is the highest, it was used to prepare solid inclusion complexes.

The effects of HPβCD at different concentrations on the ^{1}H-NMR spectrum of ABPP are shown in Fig. 3. These experiments were carried out in deuterated sodium hydroxide to make the dissolution of the drug possible. All the ^{1}H- chemical shift in the spectrum of the pure drug are located in the aromatic region (7.2–7.4 ppm) but, the shift assignment was not possible. Nevertheless, it is observed that upon complexation, the ABPP signals splits into two groups, one shifted upfield and the other downfield. These data suggest that at least part of the drug molecule interacts with the CD cavity and is encapsulated. Similar effects have been previously described in the interaction between ABPP and β-CD (Ahmed et al., 1991).

Solid inclusion complex of ABPP:HPβCD in 1:1 molar ratio was prepared by the freeze-drying method. The amount of complex that contains 150 mg of drug is 1205 mg approximately. This could be formulated even in a hard gelatin capsule or as a powder to dissolve in water before administration.

Table 1
Apparent stability constant ($K_{1:1}$) of the ABPP-cyclodextrin 1:1 complex calculated from the phase solubility diagram

Cyclodextrin derivative	$K_{1:1}$ (M^{-1})
α-CD	19.5
β-CD	350.7
γ-CD	45.5
HPβCD	461.0

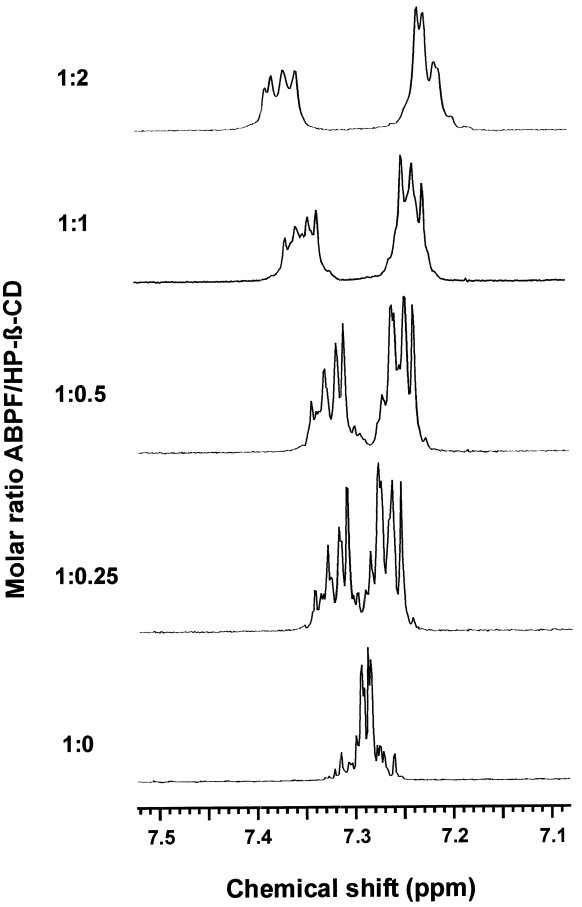

Fig. 3. ^{1}H-NMR spectra of ABPP-HPβCD inclusion complexes with the indicated different molar ratio.

A good solubilization of ABPP into water occurs upon the addition of ammonium hydroxide. This additive was used in the preparation of crystalline cyclodextrin complexes (Kurozumi et al., 1975).

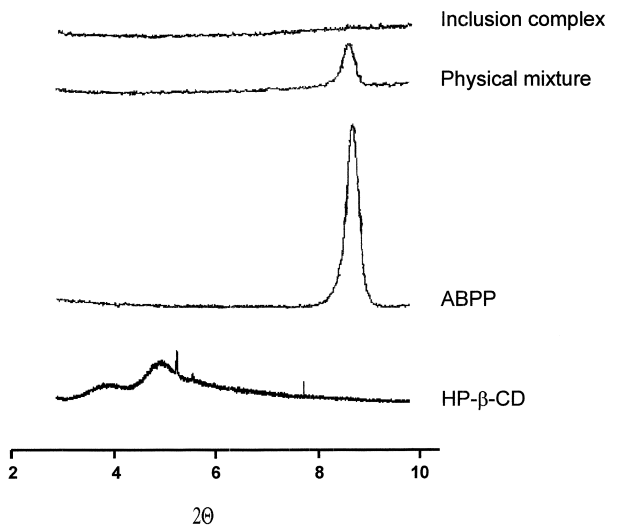

Fig. 4. Powder X-ray diffraction pattern corresponding to the indicated products.

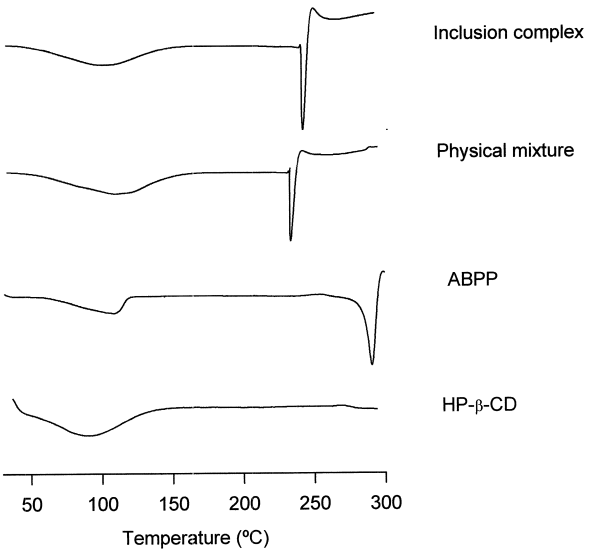

Fig. 5. Thermograms from differential scanning calorimetry (DSC) corresponding to the indicated products.

Complex of cyclodextrins with ammonia have rather high stability (Hirsch et al., 1987), but, due to the volatility of ammonia, no difficulty was encountered in its removal by freeze-drying. Freeze drying of solutions removed ammonia to an extent that it could not be detected by the Nessler reagent, and the process led to the inclusion complex of ABPP with hydroxypropyl-β-cyclodextrin.

Fig. 4 shows the X-ray powder diffraction patters of ABPP, physical mixtures (1:1 molar ratio) and the inclusion complex. Because the hydroxypropyl-β-cyclodextrin in solid state is amorphous, an eventual complexation is easily verified. In fact, the physical mixture shows the diffraction peaks corresponding to the drug, while the inclusion complex corresponds to an amorphous system and thus no evidence of the drug is present.

On the DSC curves (Fig. 5), the peaks corresponding to

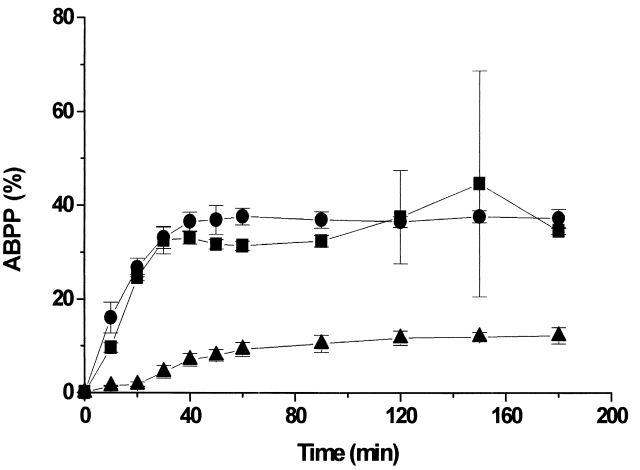

Fig. 6. Dissolution profiles of ABPP and ABPP-HPβCD inclusion complex in water at 37°C. (▲) ABPP, (■) physical mixture, (●) inclusion complex.

the evaporation of water appeared in the temperature range of 50–150°C. These peaks either decrease significantly or even disappear for the inclusion complex, which indicates that the drug penetrates into the cyclodextrin cavity replacing the water molecules (Singh et al., 1998). The thermogram of ABPP reveals an endotherm with an onset near 289°C. This develops into a peak showing irregular variations which are characteristic of a thermal degradation reaction. In the physical mixture and inclusion complex curves, the peak corresponding to the melting of the ABPP disappeared, however a new exothermic transition was observed. This exothermic event, occurring with an onset temperature of 229°C and 237°C for the mixture and the complex, respectively, has been ascribed due to the degradation of the drug in the system. The cyclodextrin facilitate thermal degradations of the ABPP, similar to that shown by PEG 20 M (Irwin and Iqbal, 1998), but the effect seems to be different when the drug is encapsulated inside the cyclodextrin.

Fig. 6 shows the dissolution profiles of ABPP, physical mixture and the inclusion complex in simulated intestinal fluid without enzymes (USP23). All systems with cyclodextrin led to an enhancement of the ABPP dissolution rate, in fact the amount of drug dissolved is up to 35% of

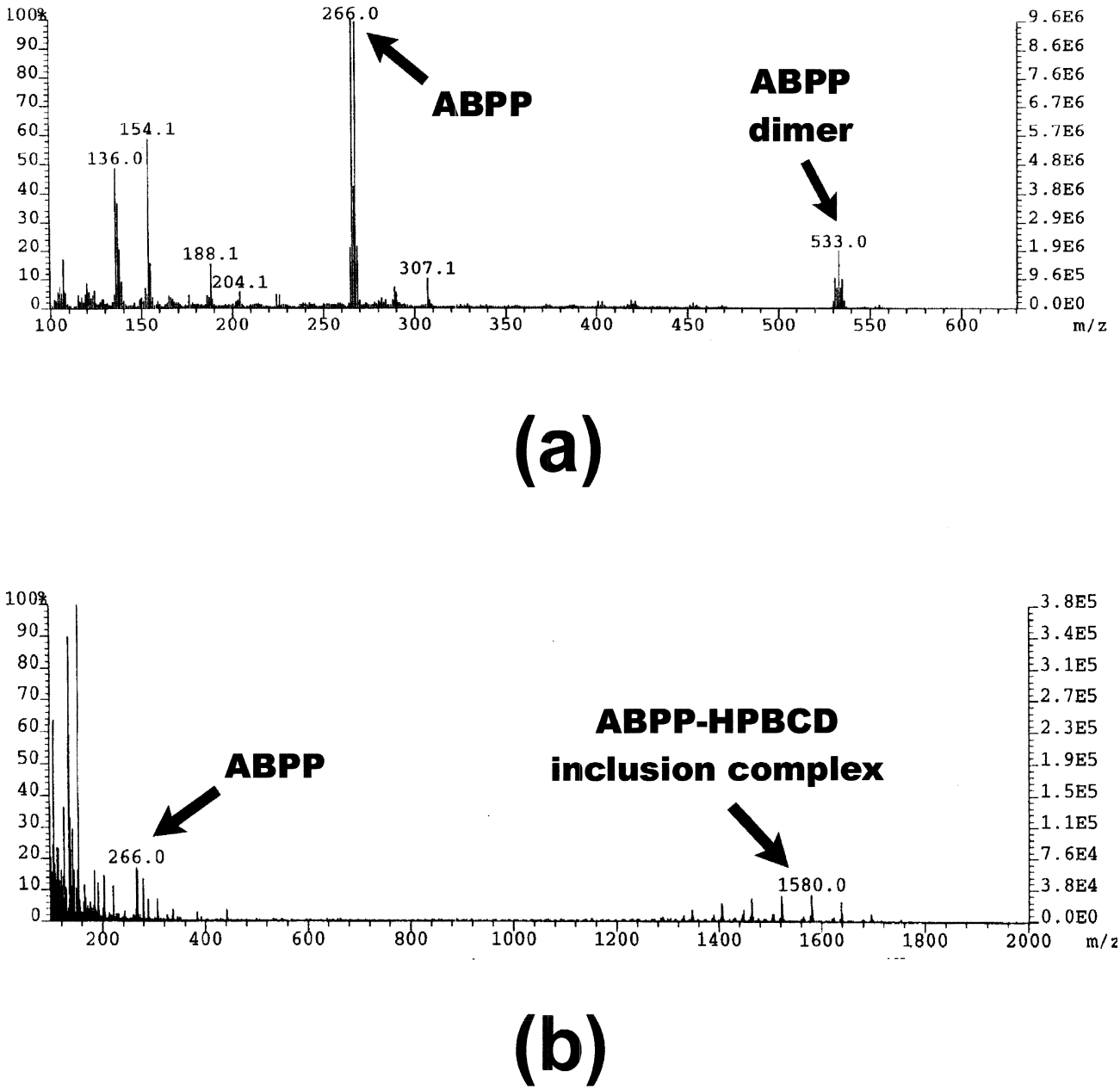

Fig. 7. FAB mass spectra of ABPP (a) and ABPP–HPβCD inclusion complex (b).

the dose (about 53 mg). This improves previous results obtained with β-CD in similar conditions but with a lower ABPP dose (19 mg) (Ahmed et al., 1993).

To characterize the dissolution profiles, dissolution efficiency was used. The model independent nature of this parameter permits the evasion of the statistical problems of kinetic constant values analysis with an associated estimation error. The ANOVA demonstrated that the factor formulation has a significant effect on 0–180 min dissolution efficiency ($F_{(2,6)}=161.59$, $\alpha<0.01$), and according to the LSD test, the systems were classified as follows (form lower to higher DE_{180}):

ABPP Physical mixture Inclusion complex

Taking into account that the dissolution behavior of the physical mixture has been found to be similar to that of the inclusion complex, we can conclude that the presence in the dissolution medium of both compounds produces a rapid complexation. The increase of the dissolution rate of the drug can be attributed to the breakdown in solution of the drug dimers in the presence of the cyclodextrin and to the complex formation. In fact, although the ABPP molecule possesses polar groups it is poorly soluble in water, presumably due to the formation of dimers (Alpar et al., 1986). Fig. 7 shows the mass spectra corresponding to ABPP (a) and the inclusion complex ABPP:HPβCD (1:1 mol:mol) (b). The spectrum of ABPP shows the peak corresponding to the drug ($M^+=266$) and the dimer ($M^+=533$). Nevertheless, only the peak of the pure drug ($M^+=266$) and the inclusion complex ($M^+=1580$) are present in the spectrum of the complex. Therefore, the complexation of ABPP avoids dimer formation, which contributes to the effect of the cyclodextrin in the increase of the solubility in water.

In conclusion, the complexation of ABPP with HPβCD enhances both the solubility and the dissolution of the drug in aqueous media. The described HPβCD complex of ABPP can be used to develop new oral formulations and is expected to improve the drug bioavailability.

Acknowledgements

The authors wish to thank the Xunta de Galicia (project PGIDT99-PXI20305B) for financial support and Janssen Pharmaceutica and Roquette-Laisa España for supplying the hydroxypropyl-β-cyclodextrin and the β-cyclodextrin, respectively.

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