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Comparative dissolution characteristics of bropirimine-β-cyclodextrin inclusion complex and its solid dispersion with PEG 6000

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Summary

An inclusion complex of bropirimine (ABPP) with β-cyclodextrin (βCD) and its solid dispersion with polyethylene glycol 6000 (PEG 6000) were prepared by the coprecipitation method. Comparative dissolution studies revealed that the solid complex exhibited a markedly faster dissolution rate compared to the PEG 6000 solid dispersions and physical mixtures in water and phosphate buffer (pH 7.4). However, the dissolution of all the test preparations was enhanced to almost the same extent in 0.1 N HCl. ^{1}H-NMR was employed to confirm the inclusion of the drug within the β-CD cavity. The high solubility and instant release of the drug were maintained upon ageing of the solid complex for 1 year, while the release of the solid dispersions decreased. TLC investigations proved that this decrease in the release rate was not due to drug degradation.

Introduction

Bropirimine (2-amino-5-bromo-6-phenyl-pyrimidin-4(3H)-one; ABPP) possesses antiviral and antitumour activities together with a novel immunomodulatory action causing enhancement of serum interferon levels (Hamilton et al., 1980). Its poor water solubility, and consequently low dissolution, hinder its formulation and clinical trials. Alpar et al. (1986) were able to overcome this problem using cosolvency and suggested an ideal injectable formula. However, presenting the drug in an effective and bioavailable oral dosage form is still problematic. From this standpoint, Irwin and Iqbal (1988) investigated the optimum formulation of bropirimine solid dispersions focusing on the thermal properties of the system. In the meantime, Ahmed et al. (1991) suggested the utility of cyclodextrin inclusion complexation as a tool for enhancing bropirimine solubility.

The rapidly growing number of papers and patents on solid dispersions and cyclodextrins and the actual pharmaceutical use of the latter motivated the idea of comparing both approaches. Accordingly, the main goal of the present work was the preparation of a bropirimine solid inclusion complex with β-CD and solid dispersions

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with PEG 6000 and the comparison of their dissolution profiles. Exploring the effect of ageing on such preparations was another objective of this study.

Materials and Methods

Materials

Bropirimine (lot no. (AI)0237-RJI-22) was kindly donated by Upjohn Co., MI, U.S.A. β-Cyclodextrin was obtained from Roquette (Lesrtrem, France) and polyethylene glycol 6000 from Aldrich (Hohenbrunn, Germany). These materials were used as received and all other reagents and solvents were of analytical grade.

Methods

Preparation of ABPP-β-CD solid inclusion complex

Both ABPP and β-CD (1:1 molar ratio) were dissolved in water with the help of a few drops of concentrated ammonia solution. The solution was driven off in a vacuum oven at 50°C for 48 h. The absence of residual ammonia was tested in the solid products using Nessler's reagent.

Preparation of ABPP-PEG 6000 solid dispersions

The same method was adopted for the preparation of 1:1 and 1:3 w/w (drug:PEG 6000) solid dispersions and for the precipitation of a sample of the drug alone. Additionally, a 1:1 w/w ABPP-PEG 6000 solid dispersion was prepared using organic solvents. The components were dissolved in 20 ml of ethanol/acetone (1:1 v/v) and the solvents were evaporated under vacuum.

Physical mixtures were prepared by simple mixing of the drug and β-CD or PEG 6000 in the same ratios as those of the solid complex and the solid dispersions. All the samples were sieved. Fractions passing through a 250 μm sieve and retained on a 125 μm sieve were used in this study.

Storage test

Samples of the drug alone, its solid inclusion complex and 1:3 w/w solid dispersion were stored for 1 year in tightly closed containers at room temperature. TLC and the dissolution profiles of these samples were investigated and compared to those of fresh samples.

Thin-layer chromatography (TLC)

Equal volumes of alcoholic solutions of the test samples, fresh and aged, containing the same drug content, were spotted on silica gel F_{254} plates (Merck). The plates were developed using a solvent system of chloroform-acetone (9:1 v/v, respectively) and the spots were visualized using a UV lamp.

Analysis of test samples

The ABPP content of each test sample was estimated spectrophotometrically (Uvedic 320, Japan) by measuring the absorbances of their solutions in water at 305 nm. Neither β-CD nor PEG 6000 interfere with the assay of the drug.

^{1}H-NMR

^{1}H-NMR spectra of the ABPP-β-CD complex and β-CD (5–7 mg/ml) dissolved in 20% ND_3 in D_20 (pH 9 buffer, Merck) were recorded with a Bruker CXP 300 Spectrometer (300 MHz). The chemical shifts were referred to the signal of residual HOD in the solvent.

Solubility of ABPP in PEG 6000 solutions

Excess amounts of the drug (10 mg) were equilibrated (48 h) by shaking with 10 ml aqueous solution of PEG 6000 (0–2% w/v) in conical flasks at 37 ± 0.5°C. Aliquots were pipetted through cotton filters and their drug contents were spectrophotometrically determined at 305 nm after dilution with water.

Dissolution test

This test was carried out on untreated ABPP, ABPP-β-CD solid inclusion complex, ABPP-PEG 6000 solid dispersion (fresh and aged samples) as well as their respective physical mixtures using a six-vessel dissolution apparatus (Erweka, DT-D6, Germany). Each sample, equivalent to 19 mg ABPP, was sprinkled on the surface of the dissolution medium (900 ml of either water, 0.1 N HCl, or low ionic strength phosphate buffer (I =

0.01) of pH 7.4 maintained at 37 ± 0.5°C and stirred at 100 rpm). At each time interval, 10 ml of the solution was withdrawn and replaced by an equal volume of the dissolution medium kept at 37°C. The concentration of ABPP dissolved was determined spectrophotometrically at 305 nm in water, 289 nm in 0.1 N HCl and at 299 nm in phosphate buffer using the same solutions as blanks. Each experiment was performed at least three times and the mean was calculated in each case.

Results and Discussion

^{1}H-NMR was chosen here to confirm the inclusion of the drug into the cyclodextrin cavity through coprecipitation. Fig. 1 and Table 1 demonstrate that the protons located within or near the cyclodextrin cavity (e.g., H-3, H-5 or H-6) were significantly more shielded than those at the exterior of the torus (e.g., H-1, H-2 and H-4). This reflects the fact that the drug molecule interacts with the interior of the torus, i.e., it is included completely or partially inside the cavity. These results are consistent with those described initially by Thakkar and Demarco (1971).

Fig. 2 and Table 2 show the dissolution characteristics, in water, of the ABPP-β-CD solid complex and its 1:1 physical mixture as well as untreated ABPP. It was found that the solid inclusion complex exhibited a markedly faster dissolution rate ($T_{50} < 2$ min) than the physical mixture ($T_{50} = 30$ min) and the untreated drug ($T_{50} = 60$ min). The dissolution of the physical mixture was relatively faster than that of the drug as a result of the effect of β-CD on the wettability and solubility of the drug (Nakai et al., 1988).

Fig. 3 presents the dissolution characteristics, in water, of ABPP-PEG 6000 solid dispersions and their corresponding physical mixtures (1:1 and 1:3 w/w ratios). It can be seen that the release rate of the 1:1 and 1:3 w/w solid dispersions is equal to or even lower than that of their equivalent physical mixtures. A possible reason for this may be that the crystallinity of the drug in the solid dispersion outweighs the wettability and the microenvironmental solubilizing action of the carrier. In accordance with this, the release of

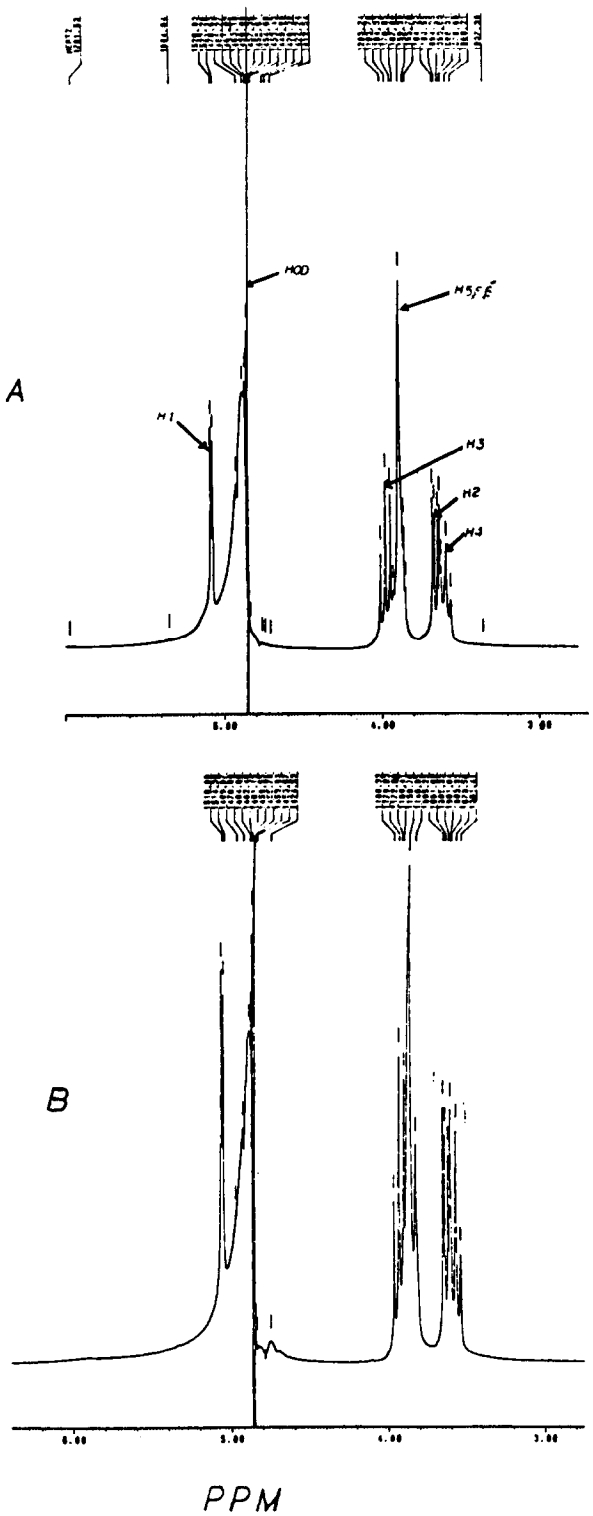

Fig. 1. ^{1}H-NMR spectra of bropirimine-β-CD inclusion complex. (A) β-CD alone; (B) ABPP-β-CD inclusion complex (1:1).

TABLE 1

Bropirimine (ABPP)-induced ^1H chemical shifts of β-cyclodextrin (β-CD)

Proton	H_z [a]
H-1	0.00
H-2	0.96
H-3	7.03
H-4	0.13
H-5	12.18
H-6	12.18

[a] H_z: difference in chemical shifts of β-CD protons in the presence and absence of ABPP, referred to H-1.

ABPP recrystallized from the same solvent as used for preparing solid dispersions was found to be slower than that of the untreated drug (Fig. 3). In addition, it was observed that PEG 6000 has a slight effect on the solubility of ABPP in water (Fig. 4). Another reason may be postulated based on the ability of ABPP to form a water-insoluble dimer (Alpar et al., 1986) which may become more pronounced in the presence of PEG 6000.

Fig. 5 illustrates the dissolution profiles of the solid inclusion complex, solid dispersions, recrystallized and untreated ABPP and its physical mixtures with PEG 6000 and β-CD in phosphate buffer solution pH 7.4. It is obvious that the solid

TABLE 2

Dissolution half-lives ($T_{50\%}$) for bropirimine (ABPP) test samples

Test samples	$T_{50\%}$ (min) [a]	
	Water	Buffer (pH 7.4)
Untreated ABPP	60	27
Recrystallized ABPP	> 120	44
ABPP-β-CD inlusion complex	< 2	< 2
ABPP-β-CD physical mixture	30	–
ABPP-PEG 6000 fresh solid dispersion (1 : 1 w/w)	50	60
ABPP-PEG 6000 fresh solid dispersion (1 : 3 w/w)	21	15
ABPP-PEG 6000 aged solid dispersion (1 : 3 w/w)	50	30
ABPP-PEG 6000 physical mixture (1 : 3 w/w)	20	10

[a] $T_{50\%}$: time (in min) required for 50% of the drug to go into solution.

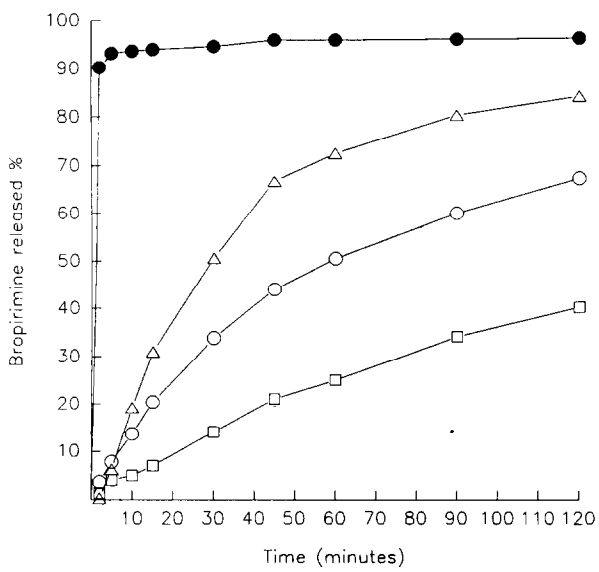

Fig. 2. Dissoltion profiles of bropirimine and bropirimine-β-CD inclusion complex in water at 37°C. (○) Untreated ABPP, (□) recrystallized ABPP, (△) physical mixture, (●) inclusion complex.

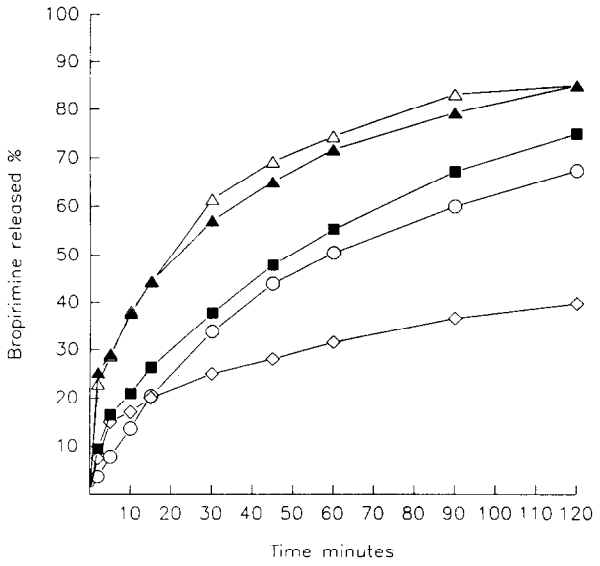

Fig. 3. Dissolution profiles of bropirimine and bropirimine-PEG 6000 solid dispersion in water at 37°C. (○) Untreated ABPP, (□) recrystallized ABPP, (■) solid dispersion (1 : 1 w/w), (▲) solid dispersion (1 : 3 w/w), (△) physical mixture (1 : 3 w/w), (◇) solid dispersion (1 : 1 w/w) from organic solvent.

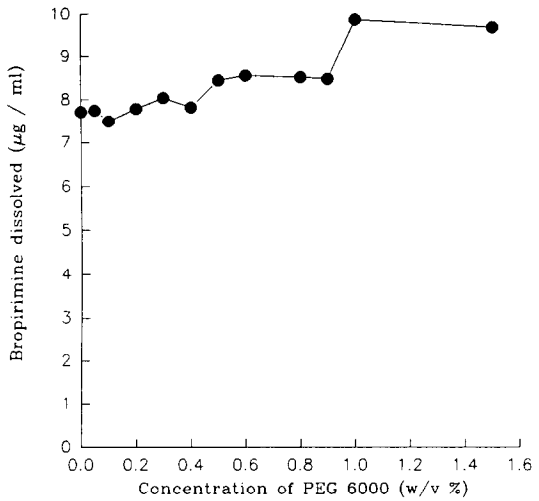

Fig. 4. Effect of pEG 6000 on the solubility of bropirimine in water at 37°C.

complex exhibited the highest dissolution rate in comparison with the solid dispersion, drug alone and the physical mixtures. Generally, the release of ABPP from the solid complex, in both water and buffer solution, was significantly greater than that from the solid dispersion, which may be due to the formation of an inclusion complex. Additionally, the capacity for dimer formation is negligible since each molecule of the drug is individually included inside the cyclodextrin cavity.

Regarding the dissolution of ABPP, its solid inclusion complex, solid dispersions and physical mixtures in 0.1 N HCl, it was found that all dissolved very rapidly and to almost the same extent. The reason for this may be the complete ionization of the drug in acidic medium (Alpar et al., 1986).

It is obvious from Figs 3 and 5 that the dissolution of the drug from the prepared solid dispersion (1:1 w/w) using ethanol/acetone solvent instead of water is still slow. Therefore, the solvent utilized during the coprecipitation process has no effect on the dissolution characteristics. This observation motivated the use of water instead of organic solvents in this respect in order to avoid contamination of the preparations by organic solvents.

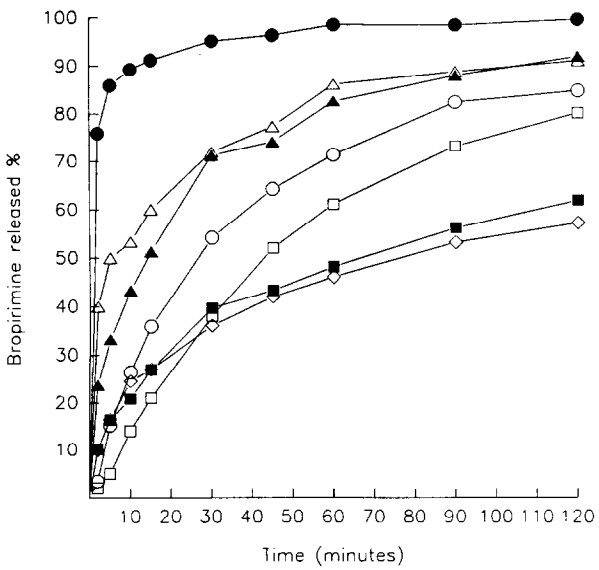

Fig. 5. Dissolution profiles of bropirimine, bropirimine-β-CD inclusion complex and bropirimine-PEG 6000 solid dispersion in phosphate buffer solution (pH 7.4) at 37°C. (○) Untreated ABPP, (□) recrystallized ABPP, (●) inclusion complex (1:1), (■) solid dispersion (1:1 w/w), (▲) solid dispersion (1:3 w/w), (△) physical mixture (1:3 w/w), (◇) solid dispersion (1:1 w/w) from organic solvent.

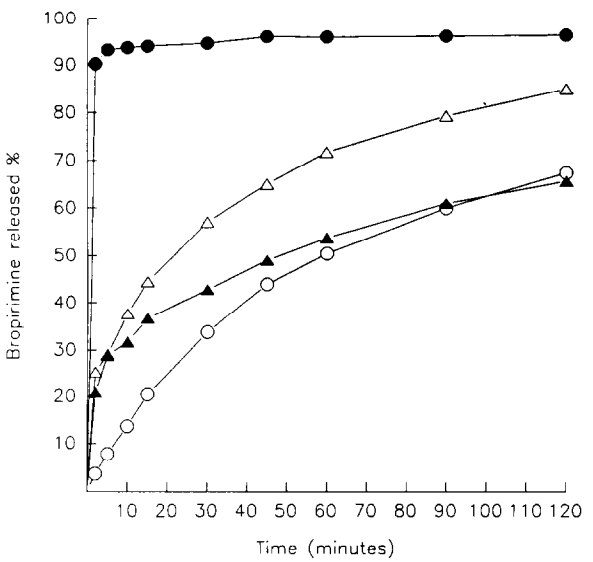

Fig. 6. Effect of ageing on the dissolution profiles of bropirimine-β-CD inclusion complex and bropirimine-PEG 6000 solid dispersion in water at 37°C. (○) Untreated ABPP, (●) aged inclusion complex, (△) fresh solid dispersion (1:3 w/w), (▲) aged solid dispersion (1:3 w/w).

Upon ageing of the solid dispersion for 1 year, a significant reduction in the dissolution rates was observed (Fig. 6 and Table 2). Unfortunately, such age-induced changes represent a considerable problem that minimizes the pharmaceutical applications of solid dispersions. Much of the literature dealt with this problem (Ford and Rubinstein, 1979; Khalil et al., 1984; Dubois et al., 1985) and in all cases these changes were attributed to the probable recrystallization of the drug due to changes in its solid solubility and its molecular state within the PEG matrix. At the same time, it is worth mentioning that the dissolution profiles of the ABPP-β-CD solid complex as well as the drug alone showed no change upon ageing for the same period. It was stated by Frömming and Hosemann (1985) that inclusion complexes have greater physical stability than solid dispersions.

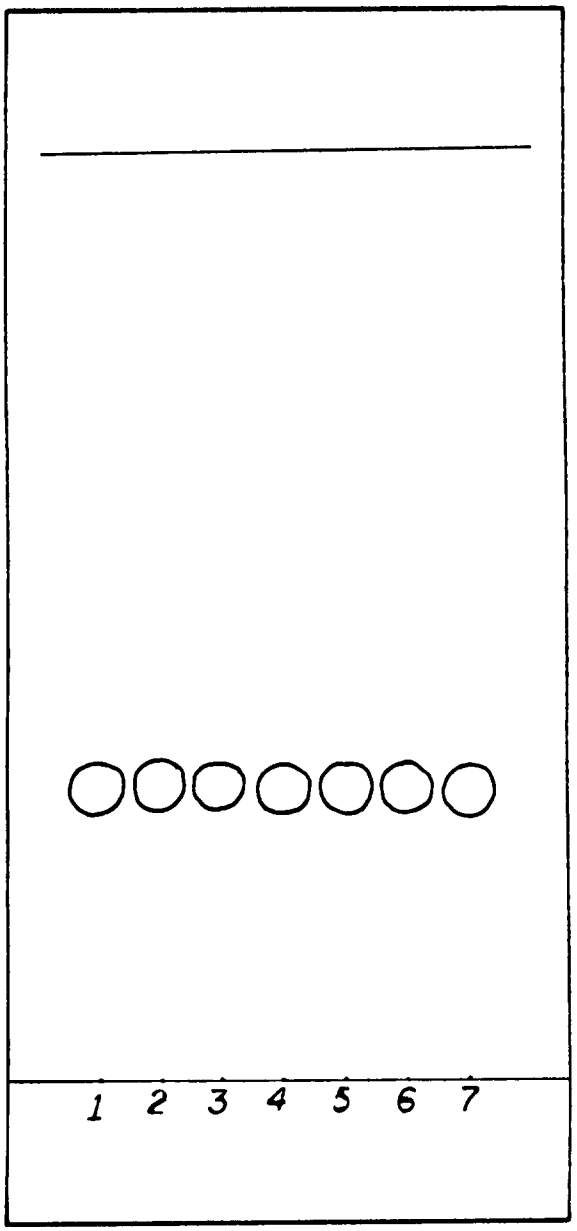

Fig. 7. TLC chromatograms of fresh and aged bropirimine-β-CD inclusion complex and bropirimine-PEG 6000 solid dispersion. (1) ABPP, (2) fresh solid dispersion (1:3 w/w), (3) fresh solid dispersion (1:1 w/w), (4) fresh inclusion complex, (5) aged inclusion complex, (6) aged solid dispersion (1:1 w/w), (7) aged solid dispersion (1:3 w/w).

Fig. 7 shows a representative TLC chromatogram for ABPP in comparison with the solid inclusion complex and the solid dispersion for freshly prepared as well as aged samples. Upon examination using a UV lamp, each sample gave a single fluorescent spot with the same R_f value ($R_f = 0.287$). This reflects the fact that neither ageing of the samples nor the method adopted for their preparation resulted in the formation of a new chemical compound. It also revealed that no chemical degradation of the drug occurred.

Conclusions

From the results obtained in this study, it could be concluded that:

The dissolution of bropirimine from its solid β-cyclodextrin inclusion complex is better than from its PEG 6000 solid dispersion.

Ageing of the solid dispersions has deleterious effects on the dissolution of bropirimine whereas such ageing has no effects on the dissolution of the drug from the prepared inclusion complex.

Generally, inclusion complexation of ABPP into β-CD could be considered a promising technique for enhancing the solubility, dissolution rate and consequently the bioavailability of this drug. This will in turn facilitate the future formulation of effective bioavailable oral dosage forms of bropirimine.

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