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Multicomponent System of Albendazole with Cyclodextrins and Hydroxyacids

R. Kalaiselvan*, G. P. Mohanta, P. K. Manna and R. Manavalan

Department of Pharmacy, Annamalai University, Annamalai Nagar, Tamil Nadu – 608 002, India.

Abstract

Ternary inclusion systems of albendazole (ABZ) with cyclodextrins and carboxylic acids in equimolar ratio were prepared using solvent coevaporation and kneading methods. Differential solubility study results showed that inclusion yields were remarkably increased by hydroxy carboxylic acids especially L-tartaric acid. The yield was higher with the coevaporation method than with the kneading method. Differential scanning calorimetric analysis report was consistent with the above findings and also showed that the free ABZ in the kneaded systems existed in the amorphous state. The higher the inclusion yield, the lower was the dehydration enthalpy of the products, signifying the complex formation. Complex formation was also investigated by phase solubility study, X-ray diffraction analysis, infrared spectroscopy and scanning electron microscopy. The dissolution rate and efficiency depended on the inclusion yield and the method of preparation. A three-fold enhancement in the bioavailability was obtained with the ABZ-HPβCD-tartaric acid ternary coevaporated system.

Key words: Albendazole, cyclodextrin, ternary complex, inclusion yield, dissolution, bioavailability

Introduction

Complexation with cyclodextrin (CD) is known as an effective method for enhancing dissolution properties and bioavailability of poorly soluble drugs. For several reasons, including toxicity, cost and dosage, the amount of CD in the dosage form has to be reduced as far as possible (Redenti *et al*,. 2000). Different approaches can be considered for achieving this goal. The first is the use of chemically modified CDs, which present a higher solubility in water (Redenti *et al.*, 2000). The second method consists in adding a water-soluble polymer like PVP or methylcellulose with the aim to increase the solubility of both the complex and the drug itself (Loftsson and Fririksdottir, 1998). The third method is the use of an acidic ternary compound. In the case of a basic drug, the acidic agent, for instance, citric acid, promotes the solubilization of the guest molecule both by forming a salt and by increasing the stability constant of the complex (Mura *et al.*, 2001). The same approach could be applied to an acidic drug by adding a basic ternary compound (Iel *et al.*, 1997). In the present study the possibility of improving inclusion efficiency of β-cyclodextrin (βCD) and hydroxypropyl β-cyclodextrin (HPβCD) in presence of some carboxylic acids (acids) was investigated using albendazole (ABZ) as a model basic drug.

Systemic absorption of ABZ is warranted for the treatment of inoperable or disseminated cases of hydatidosis, other systemic helminthiases, AIDS related microspordia, and giardiasis (Wen *et al.*, 1993; Lecuit *et al.*, 1994; Misra *et al.*, 1995; WHO, 1996; Lopez-Garcia *et al.*, 1997;

* Corresponding author : e-mail: kalaipharmacy@yahoo.com

Keramidas *et al.*, 2004). ABZ belongs to biopharmaceutical classification system type II (low aqueous solubility with high permeability), thus showing dissolution rate limited absorption (Amidon *et al.*, 1995; Jung *et al.*, 1998). The drug satisfies the structural criteria (rule 5) proposed by Lipinski *et al* (1997) in that it does not have: more than 5 hydrogen-bond donor moieties; molecular weight > 500; log P > 5; more than 10 hydrogen-bond acceptor moieties. ABZ has been reported to form a week binary complex with CDs (K_c<204 in water), which increased area under the plasma level curve (AUC) by just 44% in mice (Castillo *et al.*, 1999). The ultimate goal of this research was to produce a greater improvement in the dissolution and bioavailability of ABZ by ternary complexation with CDs and acids. In our previous studies, preparation and characterization of solid dispersions, βCD binary complexes and tablets with scale-independent dissolution were investigated for improving bioavailability of mebendazole, a drug structurally similar to ABZ (Kalaiselvan *et al.*, 2003; Kalaiselvan *et al.*, 2004).

Materials and Methods

ABZ, albendazole sulphoxide (Juggat Pharma, Bangalore, India), βCD (Ajanta Pharma Ltd., Mumbai, India) HPβCD (Natco Pharma Ltd., Hyderabad, India) and mebendazole (Cadila Pharmaceuticals Ltd., Ahmedabad, India), were gift samples. Maleic, fumaric, citric and L-tartaric acids were purchased from commercial sources (SD Fine Chem, Mumbai, India). All other materials used were of either analytical or HPLC grade.

Preparation of physical mixtures and inclusion complexes

The physical mixture (PM) was prepared by mixing ABZ, CD, and acid (1:1:0 or 1:1:1 molar ratio) in a mortar. Solvent coevaporation method was employed to obtain binary and ternary coevaporated complexes. The aqueous solution of CD was added to methanolic solution of ABZ and acid. The resulting mixture was stirred for 1 h and then the solvent was evaporated at 45°C with a rotary evaporator. The coprecipitate was passed through 30 mesh, dried under vacuum at 45°C (24 h) and passed through 60 mesh before packing in an airtight container.

Kneaded complexes were prepared by trituration of the PM using a small volume of methanol-water (1:1) solution to give a thick slurry for 45 min. The wet mass was dried at 45°C, passed through 30 mesh, dried again under vacuum at 45°C (24 h) and passed through 60 mesh before packing in an airtight container.

High Performance Liquid Chromatography

ABZ was assayed by HPLC method. A liquid chromatograph (LC-10ADVP, Shimadzu, Japan) equipped with a variable wavelength UV detector (SPD-10A, Shimadzu), a Rheodyne injector valve (model 7125) and a Supelcosil ODS analytical column (5μm, 0.46 cm × 25 i.d.) was used. The mobile phase consisted of 0.05 M phosphate buffer (pH 7.0)-acetonitrile (55:45) and the pH was adjusted to 6.5 using phosphoric acid. The flow rate was 1.0 ml/min and the detection was at 310 nm.

Determination of inclusion yield

The inclusion yields were determined in triplicate using differential solubility method (Hees *et al.*, 2002). This method includes two steps. The first involves the solubilization of the PM or the complex in a solvent that dissolves the drug, the CD and the ternary agent (acid). This step allows determining total drug content. The second step consists in the solubilization of the free (uncomplexed) drug with an organic solvent in which the CD is insoluble and in which the included drug remains entrapped. In the present study the total ABZ content was determined by dissolving an exactly known quantity of sample in the HPLC mobile phase. The ABZ contained

in the resulting solution was assayed after appropriate dilution using HPLC method described above. In the same way, the free ABZ content was measured by dispersing the sample in acetonitrile followed by sonication (5 min) and filtration through a membrane filter (0.22 μm). Determination of the total ABZ content in the products allows assessing that there is no loss of ABZ during the preparation of complexes.

Differential scanning calorimetry

Differential Scanning Calorimetry (DSC) was carried out with a heating rate of 5°C min^{-1} using a DSC 60 instrument (Shimadzu, Kyoto, Japan). The samples were scanned in triplicate. TA 60 WS software (Version 1.4, Shimadzu) was used to operate the instrument and analyse the thermogram events.

X-ray diffraction analysis

Powder X-ray diffraction (XRD) patterns were determined with an X-ray diffractometer (X'Pert MPD, Philips Electronics, Netherlands), employing CuK_{α} radiation source operating at 30 mA and 40 kV. Samples were scanned from 3 to 40° 2θ range at a scanning rate of 0.02°2θ s^{-1}. The positions and intensities of diffraction peaks were considered for the identification and comparison of crystallinity of the drug in the samples.

Infrared spectroscopy

Fourier transform infrared (FTIR) spectra were obtained on a Perkin-Elmer FTIR spectrophotometer (Spectrum one B 68718, USA) with a resolution of 2 cm^{-1} from 4000 to 400 cm^{-1}. Pellets were prepared by gently mixing the sample with potassium bromide (1:100 ratio).

Phase solubility studies

Phase solubility studies for ABZ were performed as described by Higuchi and Connors (1965). Excess amount of ABZ was added to distilled water or a hydroxy acid solution containing various concentrations of CD and shaken for 96 h at 37°C. Samples withdrawn were filtered through a membrane filter (0.45 μm), diluted and analyzed in a UV spectrophotometer at 291 nm (UV-1601 PC, Shimadzu, Japan). Solubility measurements were performed in triplicate.

Dissolution studies

In vitro dissolution studies were carried out in 900 ml of hydrochloric acid buffer medium (pH 2) or distilled water at 37°C, using USP XXIII type 2 dissolution apparatus with an agitation of 100 rpm. Powder sample equivalent to 100 mg of drug was clamped between infusion filter paper and immersed in the dissolution medium (pH 2). Sample equivalent to 25 mg of drug was also tested similarly in distilled water. The dissolution sample (5 ml) withdrawn at different time intervals was filtered through a membrane filter (0.45 μm) and assayed spectrophotometrically at 291 nm. Fresh medium was added to maintain a constant volume after each sampling. Experiments were made in triplicate.

Scanning electron microscopy

The morphology of the pure drug, ternary PM containing ABZ-HPβCD-tartaric acid and the inclusion complexes was studied using a scanning electron microscope (JSM-5610 LV Jeol, Japan). Samples were coated with platinum to provide a conductive layer for observing images at 15 kV and 800 times magnification.

Stability studies
Effect of stress condition on the stability of ABZ-HPβCD-citric acid and ABZ-HPβCD-tartaric acid systems was investigated. Samples in airtight glass containers were stored in an environmental test chamber at 40°C (75% RH). The total and free ABZ contents were determined for the ternary complexes and the PMs after 3 months. DSC and dissolution studies (in buffer medium pH 2) were also performed.

Bioavailability studies
Experimental protocols for the animal study were approved by the Institutional Animal Ethics Committee (CPCSEA / 160 / 1999). Six male Swiss albino rabbits fasted for 18 h, providing only water, were taken and administered the ternary PM, coevaporated and kneaded complexes of ABZ-HPβCD-tartaric acid combination. Each formulation was administered just once to each animal in a Latin square crossover fashion, which consisted of 3 study periods (Brahmankar and Jaiswal, 1995). Sample equivalent to 50 mg of ABZ per kg of body weight with water (20 ml) was administered. A washout period of 14 days was allowed between the study periods. Blood sample was withdrawn from the marginal ear vein at 0, 20, 40, 60, 80, 120, 180, 270, and 360 min, heparinized and centrifuged individually. The plasma was separated and freezed until analyzed.

Plasma sample analysis
To 200 µl of plasma sample in a 15 ml test tube, borate buffer (pH 10.5) (3 ml) and chloroform (4 ml) were added and mixed thoroughly using a vortex mixer (5 min). It was then centrifuged at 2,500 rpm (10 min). Chloroform layer (2 ml) was removed to another test tube and the solvent was evaporated to dryness in vacuum at 40°C. The residue in the tube was reconstituted to 100 µl (and diluted, when required) with acetonitrile containing mebendazole (1 µg/ml) as an internal standard and injected into the HPLC system.

Results and Discussion

Influence of cyclodextrins and acids on the solid complex formation
For all physical mixtures, extraction efficiencies were found to be higher than 96.2% and the inclusion yields were less than 2.3%. Since ABZ is absolutely free in PMs, the obtained inclusion values confirm that this differential solubility method is able to solubilize all the free ABZ. The maximum yield with the binary system, about 39%, was obtained with ABZ-HPβCD coevaporated complex.

Table 1 shows the influence of simple dicarboxylic acids (maleic acid and fumaric acids) and hydroxy carboxylic acids (citric and L-tartaric acids) on the ABZ inclusion. Compared to the binary systems, the yields were remarkably increased by these acids. Citric and tartaric acids almost doubled the inclusion %. Tartaric acid was observed to be more efficient than citric acid as a ternary agent ($p<0.05$), presumably due to the fact that the former has two hydroxyl groups. The highest yield, 87.42% was obtained with ABZ-HPβCD-tartaric acid coevaporated system. For all combinations, the kneading method showed significantly lower inclusion yield ($p<0.05$).

Table 1. ABZ inclusion yield influenced by the CD and acid types, and the method of preparation.

Complex type	Acid used	Cyclodextrin	Inclusion yield ±s.d. (%)	
			Kneaded complex	Coevaporated complex
Binary system	--	βCD	17.22±2.08	24.52±1.53
		HPβCD	28.18±2.81	38.57±3.08
Ternary system	Maleic acid	βCD	22.26±1.38	30.11±1.36
		HPβCD	31.12±2.88	44.66±2.41
Ternary system	Fumaric acid	βCD	27.51±1.10	36.59±2.22
		HPβCD	49.02±3.63	62.31±2.89
Ternary system	Citric acid	βCD	30.15±1.04	44.01±2.15
		HPβCD	61.52±3.00	74.83±4.01
Ternary system	Tartaric acid	βCD	40.18±2.91	50.84±3.06
		HPβCD	71.21±4.44	87.42±5.10

In order to estimate the effect of the acid conformation, the influence of maleic and fumaric acid was evaluated. When the double bond conformation was *cis* (maleic acid), the interaction was relatively weaker than when the conformation was *trans* (fumaric acid) ($p<0.05$). For instance, the inclusion yield with the ABZ-HPβCD-maleic acid coprecipitate was only 44.66%, while it was 62.31% with ABZ-HPβCD-fumaric acid system. This kind of molecular interaction has been described previously for ternary complexes between pyrene, βCD and several alcohols (Munos de la Pena *et al.* 1991). Hydroxy acids produced significantly higher inclusion efficiency than the simple dicarboxylic acids ($p<0.05$).

The thermal behavior of inclusion complexes was investigated using DSC to confirm the enhancement of inclusion efficiency. Typical DSC thermograms of the binary and ternary complexes are shown in Fig. 1. The thermogram of ABZ exhibited a melting peak at 200°C. βCD showed a broad endotherm centered at 110°C and HPβCD at 119°C, which may be attributed to the dehydration of the water within the CD cavity. Complex formation was suggested by a decrease in enthalpy of dehydration when compared to the PM. As the bound water molecules of CD cavity are replaced with the drug molecules during complexation, the resultant dehydration enthalpy is relatively less (Loftsson and Brewster, 1996; Moore *et al.*, 2000). The enthalpy value for the ternary PM of ABZ-HPβCD-citric acid was 656.03 Jg^{-1}. Whereas the corresponding coevaporated and kneaded products showed 164.86 and 269.72 Jg^{-1} respectively. The coevaporated system showed relatively less dehydration enthalpy due to higher inclusion, which is in confirmation with the results obtained with the differential solubility technique.

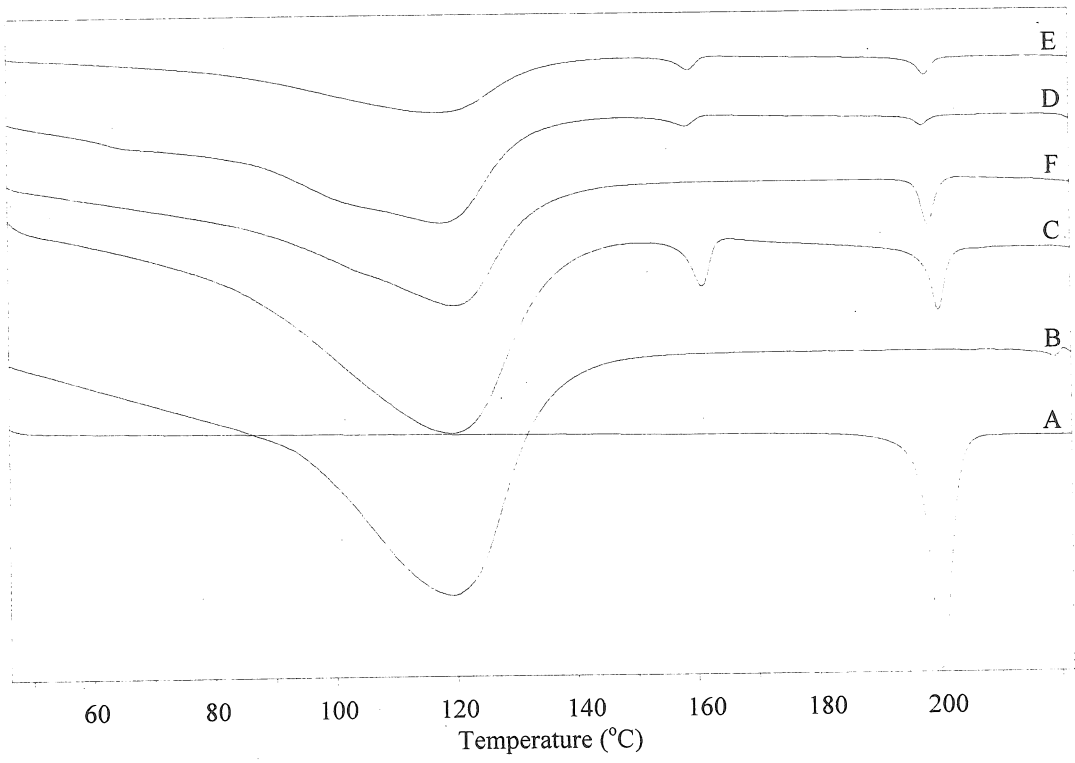


Figure 1: DSC thermograms of HPβCD systems. (A) ABZ, (B) HPβCD, (C) ternary PM with citric acid, (D) kneaded ternary system, (E) coevaporated ternary system, (F) coevaporated binary system

Interestingly, the kneaded complexes showed a weak drug-melting peak indicating the existence of free drug in amorphous form. XRD patterns of coevaporated ternary systems showed reduced diffraction intensity only for the drug and acid, whereas the kneaded products showed intensity reduction for the entire complex.

Molecular interaction

The specific role of hydroxy acids in the inclusion complex formation was investigated by FTIR. Typical FTIR spectra for the HPβCD systems are shown in Fig. 2. The spectrum for ABZ has NH stretching vibration at 3323 cm^{-1} due to carbamate-NH, whereas the broad band centered at 2665 cm^{-1} is due to imidazole-NH (intramolecular hydrogen bonded with carbamate carbonyl [1717 cm^{-1}] to form a stabilized six membered ring) (Fig. 3A). These bands appear unchanged in the binary and ternary PMs. Binary complex shows a slight increase in the frequency of carbamate-NH stretch (by 15 cm^{-1}). In our previous study, this NH was found to be involved in hydrogen bonding with poly(vinyl pyrrolidone) (Kalaiselvan *et al.*, in press). However the same band disappears in the spectrum of ternary complexes. The broad band at 2665 cm^{-1} gets reduced in intensity, it is shifted to higher frequency and merged with CH stretch (2958 cm^{-1}). With these limited data, the proposed orientation of the drug molecule for the ternary complex formation is given in Fig. 3B.

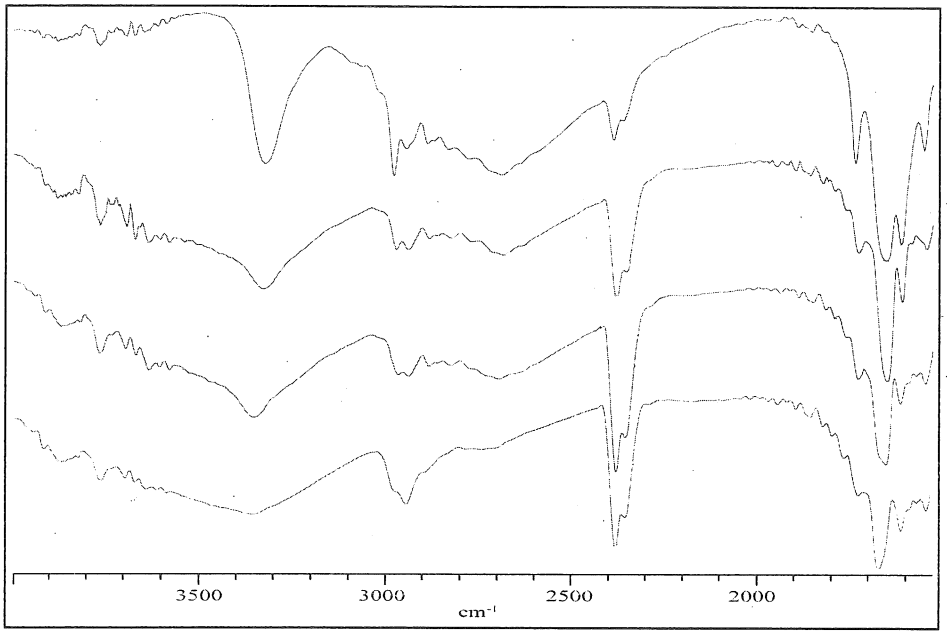


Figure 2: FTIR spectra of HPβCD systems. (A) ABZ, (B) ternary PM with tartaric acid, (C) coevaporated ternary system, (D) coevaporated binary system.

The acid forms hydrogen bond with the imidazole-NH to allow carbamate moiety transforming to enolic form (another intramolecular hydrogen bonded orientation). This form of drug is more susceptible to inclusion into the CD cavity without any steric hindrance. In the previous study, interaction of tartaric acid with the imidazole function of ketoconazole as well as with the hydroxyl group of the βCD was reported (Redenti *et al.*, 1999). In this case, hydroxy acid does not fit into the CD cavity but remains outside, while ABZ forms an inclusion complex through the interaction between its carbamate moiety and the CD cavity. By bridging the guest and the host, the acid has two interaction points. It forms hydrogen bond with the imidazole-NH of ABZ as well as hydroxyl group of the CD. Electrostatic interactions between the acid and the imidazole ring of the drug may also take place

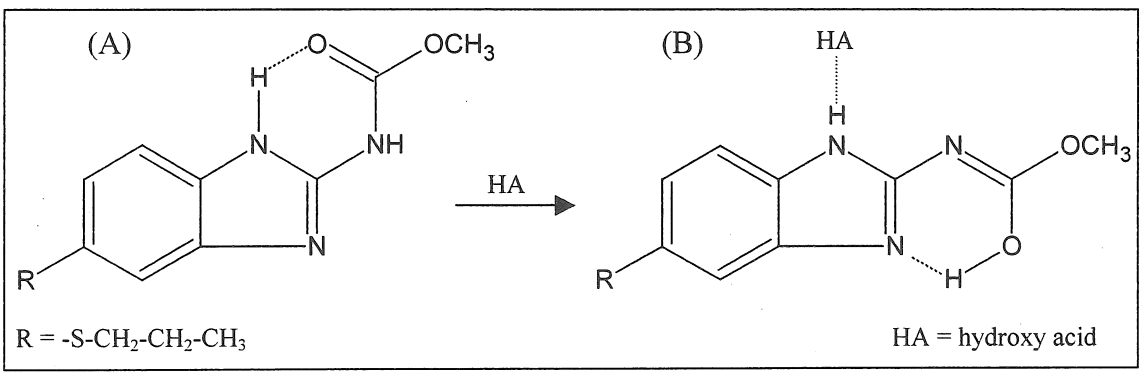


Figure 3: Chemical structure of ABZ. Keto form (A) changing to enolic form (B) due to hydrogen bonding with the acid.

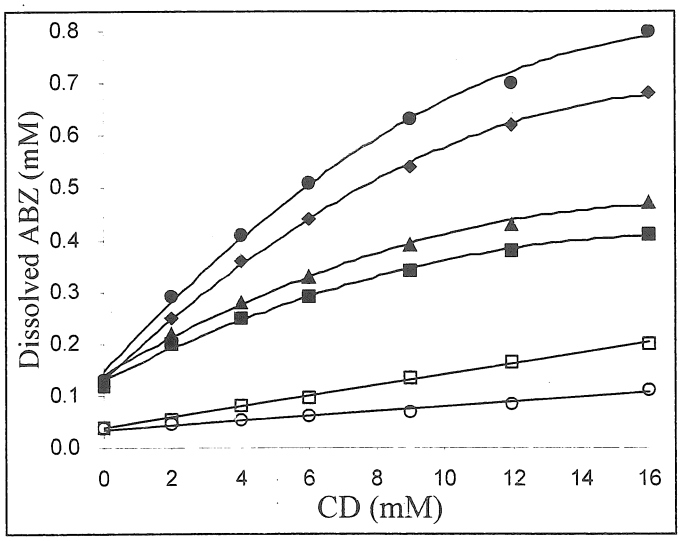
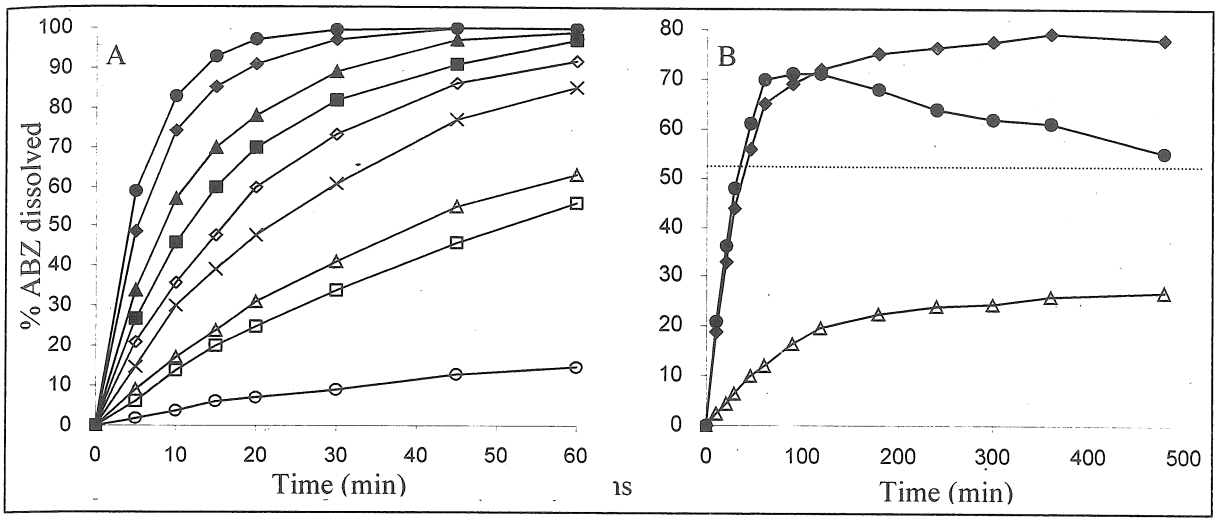


Figure 4: Phase solubility of ABZ. Solubility in presence of βCD (○) or HPβCD (□) in water; βCD (■) or HPβCD (◆) in 10 mM citric acid; βCD (▲) or HPβCD (●) in 10 mM tartaric acid

Phase solubility

Fig. 4 illustrates the phase solubility of ABZ in distilled water at 37°C in presence of CDs and acids. The intrinsic solubility of ABZ in water was very low (0.037 mM). The solubility increased as a function of the CD concentration. The solubility diagram can be classified as an A_L type in absence of acids (Higuchi and Connors, 1965). Stability constants (K_c) of 119.4 M^{-1} and 284.0 M^{-1} were obtained for ABZ-βCD and ABZ-HPβCD systems respectively. These values indicate only a weak association-dissociation. The K_c values between 200 and 5000 M^{-1} are usually considered as suitable for the bioavailability improvement (Castillo *et al.*, 1999).

In 10 mM citric or tartaric acid solution, the solubility isotherm showed a negative deviation from linearity. This isotherm is of type A_N, which is generally attributed to a change in the solute-solvent interaction like an ionization of the guest molecule (Higuchi and Connors, 1965). The value of K_c cannot be calculated from this solubility profile. A portion of 16 mM HPβCD increased the water-solubility of ABZ about 5.5 times only. Whereas a solubility rise of about 21 folds was obtained with the same amount of HPβCD in 10 mM tartaric acid solution. Tartaric acid (10 mM) alone showed only 3.2 fold solubility enhancement. Previous studies have shown that the combined effect of the acid and CD on the solubility of lipophilic drugs is due to a multicomponent complex formation (Ventura *et al.*, 1995; Mura *et al.*, 2001).



distilled water. Pure drug (○), ternary PM with citric acid (□), with tartaric acid (Δ), binary coevaporated complex (×), binary kneaded complex (□), ternary coevaporated complex with citric acid (■), with tartaric acid (◆), ternary kneaded complex with citric acid (▲), with tartaric acid (●), solubility of ABZ in a solution of 0.015% HPβCD and 0.0016 % tartaric acid (....).

Dissolution

The profiles illustrating dissolution of HPβCD systems in hydrochloric acid buffer medium (pH 2) are presented in Fig. 5A. Dissolution efficiency (DE_{60}) and time required to release 90% of drug (t_{90}) were calculated by the method of Khan (1975) (Table 2). In the present study, the powder sample was clamped between infusion filter paper and immersed in the dissolution medium. This experimental setup prevents separation of drug particles from the PM and allows formation of a hydrodynamic layer of HPβCD surrounding the ABZ particles in the dissolving microenvironment. Hence the physical mixtures also showed faster dissolution than the ABZ alone. All inclusion complexes had significantly higher DE_{60} and lower t_{90} values than the corresponding PMs and pure drug ($p<0.05$).

Table 2. Dissolution characteristics of HPβCD systems

Formulations	T_{90}±s.d. (min)	DE_{60}±s.d. (%)
Pure drug (ABZ)	558.2±60.2	8.9±0.8
Binary physical mixture (ABZ-HPβCD)	248.3±29.1	19.7±2.2
Ternary physical mixture (ABZ-HPβCD-citric acid)	154.8±21.1	32.0±4.1
Ternary physical mixture (ABZ-HPβCD-tartaric acid)	125.9±15.3	38.2±3.9
Binary coevaporated system (ABZ-HPβCD)	69.9±6.5	55.6±5.8
Binary kneaded system (ABZ-HPβCD)	50.3±4.4	64.5±5.8
Ternary coevaporated system (ABZ-HPβCD-citric acid)	37.7±2.9	71.8±5.5
Ternary kneaded system (ABZ-HPβCD-citric acid)	28.7±2.3	78.3±6.3
Ternary coevaporated system (ABZ-HPβCD-tartaric acid)	18.2±0.9	86.4±4.3
Ternary kneaded system (ABZ-HPβCD-tartaric acid)	13.0±0.78	90.9±5.4

Ternary complexes showed relatively larger dissolution rate and efficiency than the binary systems ($p<0.05$). Though the kneaded systems exhibited relatively low inclusion efficiency, they showed slightly faster dissolution than the coevaporated products ($p<0.05$). According to the DSC and XRD reports, the kneading process might have reduced the crystallinity of the free drug as well as the complex formed. However, for the given method of complex preparation, the dissolution rate and efficiency depended on the inclusion efficiency. For example, the kneaded complexes can be ranked according to the dissolution rate as ABZ-HPβCD-tartaric acid > ABZ-HPβCD-citric acid > ABZ-HPβCD binary system ($p<0.05$). The inclusion efficiency of these complexes was also in the same order.

According to Pedersen (1997), the increased bioavailability of cyclodextrin complexes is due to dissolution enhancement and supersaturation of the drug. In order to investigate the drug supersaturation event, dissolution studies in distilled water were also conducted for the kneaded and coevaporated ABZ-HPβCD-tartaric acid systems. ABZ from both products, dissolved over its solubility with a post peak decline after certain period of time (Fig. 5B). This is indicative of supersaturation caused by amorphous drug (Pedersen, 1997). The duration of the supersaturation episode for the kneaded product was remarkably low due to precipitation of high-energy (amorphous) drug molecules. In our previous study, the stability of amorphous ABZ was investigated (Kalaiselvan *et al.*, in press).

Accelerated stability studies on ABZ-HPβCD-citric acid and ABZ-HPβCD-tartaric acid systems showed no significant change in inclusion efficiency and dissolution rate (buffer pH 2) after 3 months of storage (40°C, 75% RH).

Morphology

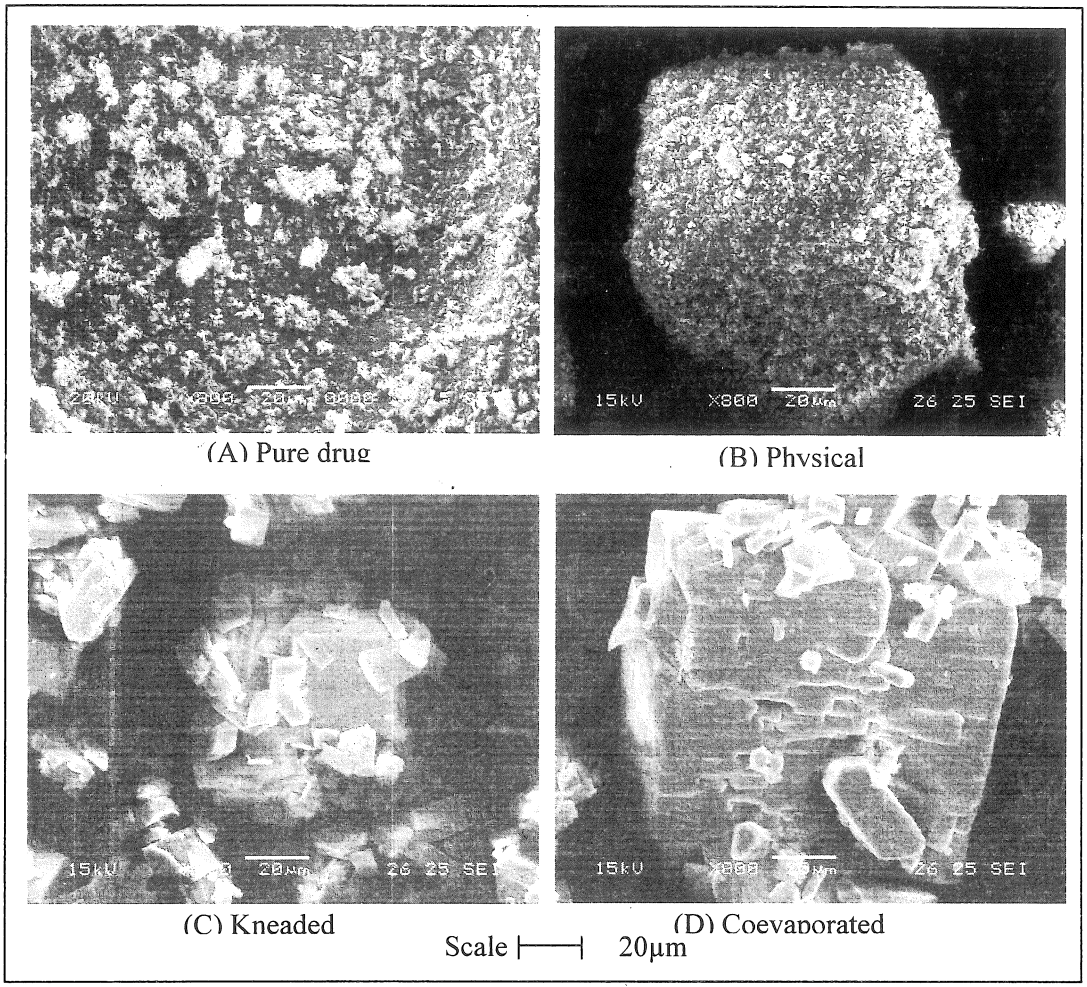


Figure 6: SEM images of HPβCD systems. ABZ (A), ternary PM with tartaric acid (B), kneaded (C) and coevaporated systems (D).

The morphology of the pure drug, ternary PM containing ABZ-HPβCD-tartaric acid and the inclusion complexes were studied. The representative photographs are shown in Fig. 6. Pure drug particles were very small in size with reduced effective surface area due to agglomeration. They remained dispersed and physically adsorbed on the surface of HPβCD as well as tartaric acid particles in the PM. Photograph in Fig. 6B shows drug particles distributed on the surface of a HPβCD particle. Both kneaded and the coevaporated systems showed a homogeneity, signifying the inclusion complex formation. The kneaded system was of poor crystal structure, lacked distinct crystal faces and had numerous cracks and fissures. This might have also contributed to the faster dissolution compared to the coevaporated system.

Bioavailability of drug from ternary complexes:

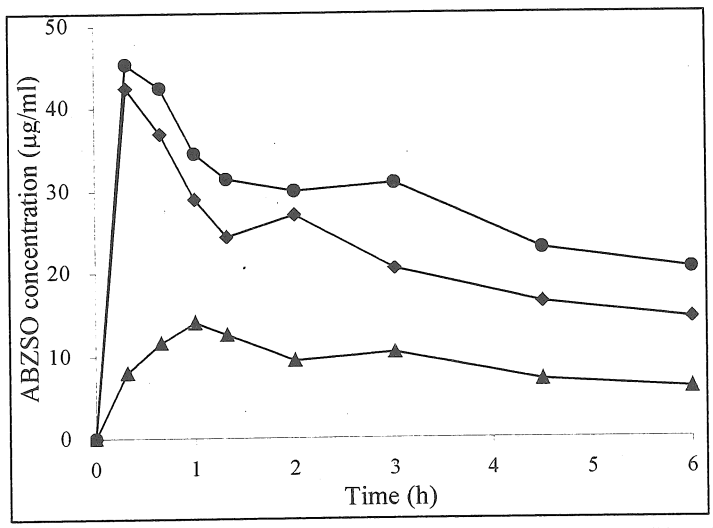


Figure 7: Mean ABZSO plasma concentration-time profile of HPβCD systems. Ternary PM with tartaric acid (▲), kneaded (◆) and coevaporated ternary complexes (●).

Table 3. Pharmacokinetic parameters of HPβCD systems

Formulations	C_{max}±s.d. (µg/ml)	T_{max} ±s.d. (h)	AUC±s.d. (µg.h/ml)
Ternary physical mixture	14.1±1.25	1.00±0.21	53.5±4.89
Ternary kneaded system	42.5±2.30	0.39±0.14	132.2±7.51
Ternary coevaporated system	45.5±2.75	0.39±0.14	170.2±8.44

After oral administration, unmodified ABZ was not detectable in any plasma sample. This is a consequence of hepatic first-pass metabolism, which is in accordance with the results previously obtained in different animal species (McKellar and Scott, 1990; Delatour *et al.*, 1991; Lopez-Garcia *et al.*, 1998). In the present bioavailability studies, the active metabolite, albendazole sulphoxide (ABZSO) was evaluated. A greater therapeutic effect was previously reported against the systemic phases of *Trichinella spiralis* in mouse with a higher plasma level time profile of ABZSO following oral administration of ABZ with methimazole (Lopez-Garcia *et al.*, 1998).

Plasma concentration time profiles of ABZSO obtained after a single dose (50 mg/kg) administration of the ABZ-HPβCD-tartaric acid ternary complexes to rabbits are shown in Fig. 7 and the different pharmacokinetic parameters are given in table 3. An increased GI absorption of ABZ from the coevaporated system was evident from the significantly reduced T_{max} and tripled C_{max} and AUC values compared to the PM ($p<0.05$). The kneaded product also showed similar increase in bioavailability except that it had a slightly low AUC ($p<0.05$). According to Pedersen (1997), the prolonged supersaturation during the dissolution process was reflected in significantly high AUC for the coevaporated system.

In conclusion, the ternary complexes exhibited a greater improvement in inclusion efficiency and hence dissolution compared to the binary systems and the physical mixture. The complex containing increased content of included drug (ABZ-HPβCD-tartaric acid coevaporated system)

showed superior supersaturation and *in vivo* bioavailability. Due to the amorphous nature, the kneaded ABZ-HPβCD-tartaric acid system exhibited a slightly higher dissolution rate compared to the coevaporated product. However, the amorphous drug precipitated when dissolved over its solubility consequently obtaining a relatively low plasma level AUC.

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