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Correlation and *in vitro* studies on radioactive and nonradioactive albendazole-ß-cyclodextrin complex tablets

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The work aims to confirm the complexation of albendazole (ABZ) by β-cyclodextrin (β-CD), and to compare them with pure ABZ tablets using radioactive and nonradioactive dissolution studies. The complex tablets were prepared by kneading a binary mixture of ABZ and β-CD and a direct compression method. Nuclear magnetic resonance (NMR) spectroscopy, scanning electron microscopy (SEM) and Fourier transform infrared (FTIR) spectroscopy were examined to prove the formation of complexes in the final products. The radiolabelled tablets were labelled with ^{99m}Tc-DTPA. Dissolution studies were performed with radiolabelled and nonradiolabelled tablets in two dissolution media (pH 1.2 and pH 7.4). The tablets were added to an acidic solution (pH = 1.2) to quantify the concentration of the drug inside the β-CD cavity. The other medium (pH = 7.4) was used to prove the existence of non-complexed drug in each powder, as the drug's solubility increases with pH. It was observed that complexation occurred in all tablets, and β-cyclodextrin (β-CD) could increase the aqueous solubility. Further, a correlation was shown between dissolution results for radiolabelled and nonradiolabelled tablets. This study shows that the characterization studies were a good indicator for the ABZ: β-CD complex. According to the phase solubility studies, the solubility of ABZ increased when the amount of β-CD increased, and drug release from tablets in pH 7.4 and pH 1.2 media was dramatically improved by the addition of β-CD compared with the pure ABZ tablet.

1. Introduction

The therapeutic activity of drugs is related to their solubility in water. To improve the solubility of a drug, various techniques can be considered namely micronisation, nanosizing, microemulsification, formulation in an amorphous solid form (e.g. solid dispersions by melt extrusion) or the modification of the physicochemical properties of the drug (e.g. salt formation or formation of water soluble complexes).

Benzimidazole carbamates are antihelmintic drugs widely used in the treatment of intestinal and tissue nematode infections and, at higher doses, for therapy of echinococcosis. ABZ is effective against infections with gastrointestinal nematodes including mixed infections of Ascaris, Trichuris and hookworms, and is the drug of choice for treating helminthiasis including trichuriasis (whipworm infections), ancylostomiasis (hookworm infections) and ascariasis (roundworm infections). It is a white to off-white powder, which is insoluble in water, soluble in dimethylsulfoxide, strong acids and strong bases, and slightly soluble in methanol, chloroform, ethyl acetate and acetonitrile (Morin et al. 2000; Castillo et al. 1999; Villaverde et al. 1992; James et al. 1996).

CDs are a group of structurally related natural products formed during the bacterial digestion of cellulose. These cyclic oligosaccharides consist of (alpha-1,4)-linked alpha-D-glucopyranose units and contain a somewhat lipophilic central cavity and a hydrophilic outer surface. α-, β-, and γ-CDs are naturally occurring CDs combining six, seven or eight glucopyranose units (Tang et al. 2002). CDs are able to form dynamic molecular inclusion complexes with many drugs, by incorporating the drug molecule, or, more commonly, a lipophilic moiety of the molecule, into the central cavity. Also, they have recently been recognized as useful pharmaceutical excipients, due to their potential to form inclusion complexes with appropriately sized drug molecules. The resulting complexes generally offer various physicochemical advantages over the free drug, including increased water solubility, enhanced bioavailability, improved stability, and reduced side effects (Mura et al. 1999; Szejtli 1988; Duchene et al. 1987). Consequently, the antihelmintic activity of ABZ can be improved by complexation with natural cyclodextrins. Among these cyclodextrins, β-CD appears to be especially useful, based on its safety in humans and its complexation potential (Moore et al. 2000; Zerrouk et al. 1998). ABZ was selected because of its low solubility and hence potential improvement in its bioavailability from an increase in its solubility.

Gamma-scintigraphy is applied extensively to the development and evaluation of pharmaceutical drug delivery systems. It is used particularly for monitoring formulations in the gastrointestinal and respiratory tracts and provides information on the deposition, dispersion and movement of the formulation. A drug formulation or base compound can be radiolabelled with a suitable gamma emitter without altering its characteristics. Also, radiolabelled drug delivery systems can be tested *in vitro* using various techniques designed to study drug release (Davis et al. 1992; Perkins and Frier 1996).

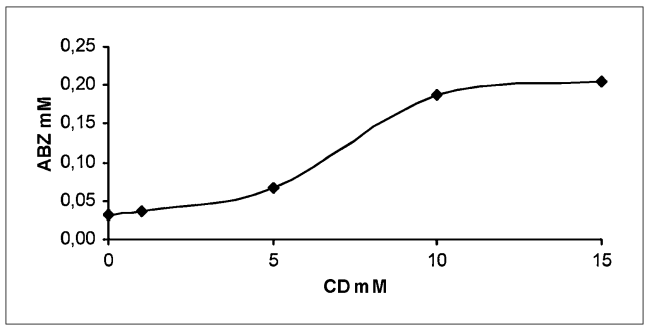

Fig. 1: Phase solubility diagram of ABZ with β-CD at 37 °C and pH 7.4

The aim of this work is to provide evidence about the yield of complex formation between β-CD and ABZ prepared by a kneading method, assess the effect of β-CD on ABZ solubility characteristics and its effect on the dissolution of tablets with and without ^{99m}Tc-DTPA radiolabelling.

2. Investigations, results and discussion

2.1. Results of phase solubility studies

The phase solubility studies showed that the concentration of ABZ in pH 7.4 phosphate buffer at 37 °C was notably affected by the presence of β-CD. The results are reported in Fig. 1. It was observed that the apparent solubility of ABZ increased as a function of β-CD concentration over the entire concentration range studied. However the solubility of ABZ was improved by increasing the concentration of β-CD from 1 mM to 10 mM, while between concentrations of 10 and 15 mM complex formation remained constant. The apparent stability constant Kc of ABZ with different ratios of β-CD in phosphate buffer medium was calculated (Kc = 213.221 M^{-1}, $r^2 = 0.9939$). The stability constant is correlated with the interaction forces between the drug and β-CD. At concentrations of β-CD between 1 mM and 10 mM, the product showed a linear increase in drug solubility with increasing β-CD concentration, thus supporting complex formation.

2.2. FTIR spectroscopy results

FTIR spectra of ABZ, β-CD, and their complexes in the 4000–3000 and 2000–1500 cm^{-1} regions (selected as the most interesting to highlight possible drug–carrier solid-state interactions) are shown in Figs. 2 and 3. The FTIR spectrum of pure ABZ shows two typical bands at 3350 and 2950 cm^{-1} relative to the N–H primary stretching vibration, and characteristic bands

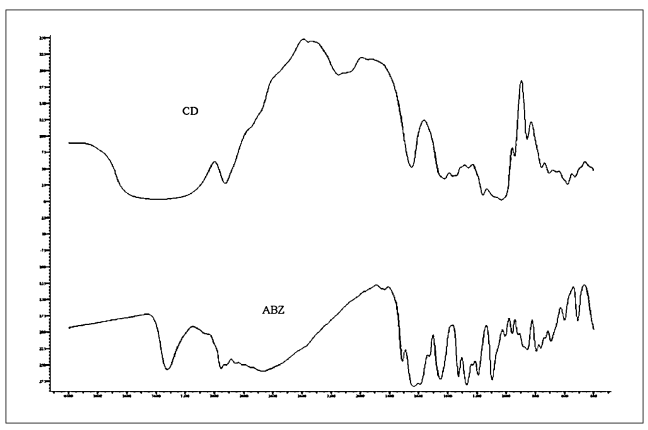

Fig. 2: FTIR spectra of ABZ and β-CD

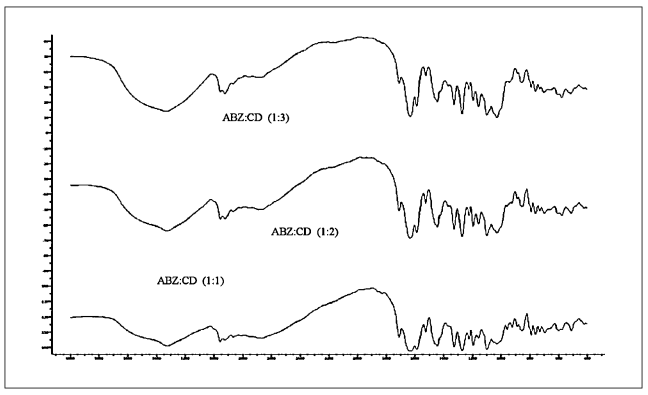

Fig. 3: FTIR spectra of ABZ:β-CD complexes

at 1626 and 1567 cm^{-1} assigned to C=N stretching. β-CD displayed a very strong band at 1741 cm^{-1} due to the C=O vibration of the acetyl group. The FTIR spectra of the ABZ:β−CD complexes products presented appreciable shifts and reduction in intensity of the characteristic ABZ bands, providing evidence for the presence of more or less intense solid-state interactions between the components, as shown in Fig. 3.

2.3. ^{1}H NMR spectroscopy results

The spectra of complexes produced with 1:1, 1:2 and 1:3 w/w ratios differed from those of the physical mixture. The differences in NMR results suggest that when the β–CD ratio is increased, the intensity of drug signals decreases. Furthermore, we observed a decrease in the intensity of the N-H primary stretching vibration at 11.5 ppm. The NMR results for β–CD show no peaks for an aromatic phase, whereas when it was complexed with ABZ the aromatic phase spectrum changed. There is an additional peak at approximately 6 ppm, corresponding to the formation of the ABZ: β–CD complex. Moreover, we observed that when the ratio of β-CD increased, the intensity of this additional pick also increased. The other vibrations seen with the complexes shifted to the aliphatic phase depending on the increased ratio of β-CD.

2.4. Scanning electron micrographs (SEM)

SEM analyses were performed on pure ABZ and β-CD samples and on their complexes prepared by a kneading method, to study possible morphological changes caused by the different treatments. ABZ particles appeared as lamellar, rather irregular-sized crystals, with a tendency to self-agglomerate (Fig. 4 B). On the oher hand, β-CD consisted of homogeneous small crystals (Fig. 4 A). The complex products differed in morphology from ABZ and β-CD (Fig. 4 C, D, E). When β-CD is kneaded with ABZ, the cavities in the β-CD are filled by molecules of ABZ. This is evidence of interaction between ABZ and β-CD in complexes.

2.5. Dissolution studies with nonradiolabelled tablets

Tablets were subjected to *in vitro* drug release studies in 0.1 N HCl and in pH 7.4 phosphate buffer. The dissolution profiles of the complexes in 0.1 N HCl and in pH 7.4 phosphate buffer are shown in Figs. 5 and 6. The release rate profiles of pure ABZ, 1:1, 1:2, and 1:3 ABZ: β-CD complex tablets were determined under sink conditions. The high ABZ dissolution obtained with the highest concentration of 1:3 ABZ:β–CD was probably because most of the ABZ molecules were surrounded by

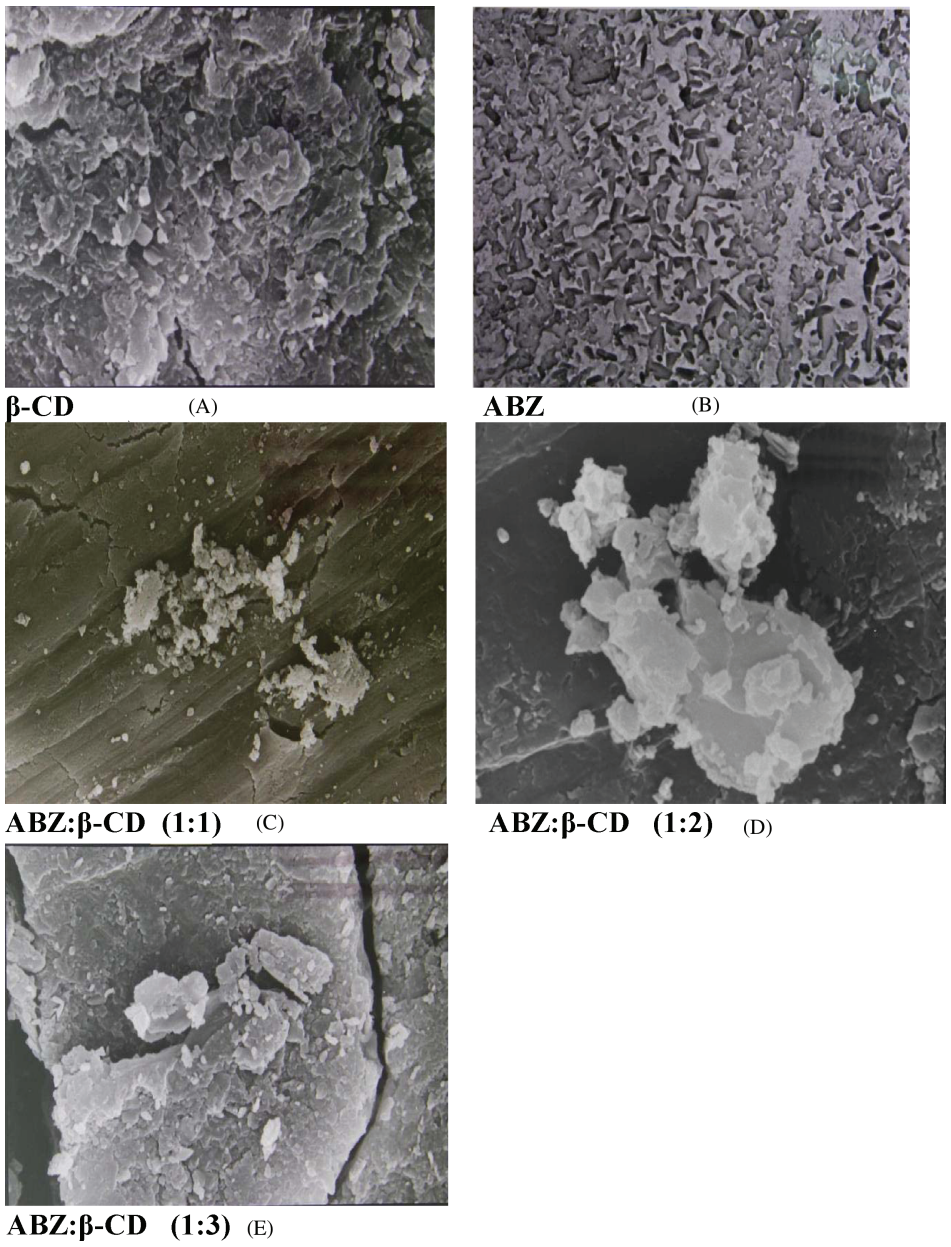

Fig. 4: Scanning electron micrographs of ABZ, β-CD and their complexes

β–CD molecules. This factor increased the hydrophilic characteristics of ABZ, and also improved its solubility. The complexes showed a rapid dissolution rate in 0.1 N HCl (pH 1.2) due to its acidic nature. In pH 7.4 phosphate buffer, they showed a slower dissolution rate than in 0.1 N HCl.

2.6. Dissolution studies with radiolabelled tablets

The dissolution profiles of radiolabelled ABZ:β-CD complex tablets in 0.1 N HCl and in pH 7.4 phosphate buffer are shown

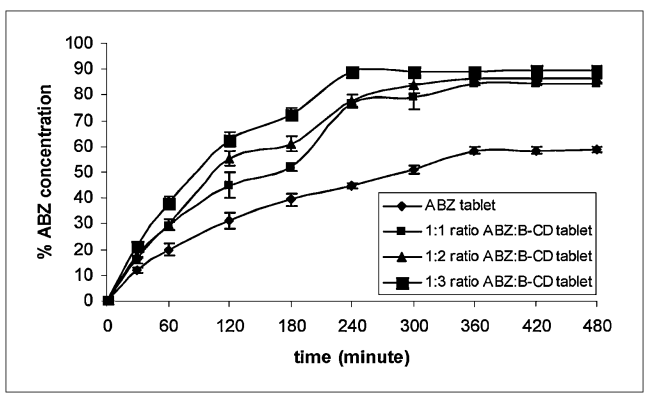

Fig. 5: Dissolution profiles of pure ABZ and ABZ: β-CD complex tablets at pH 1.2

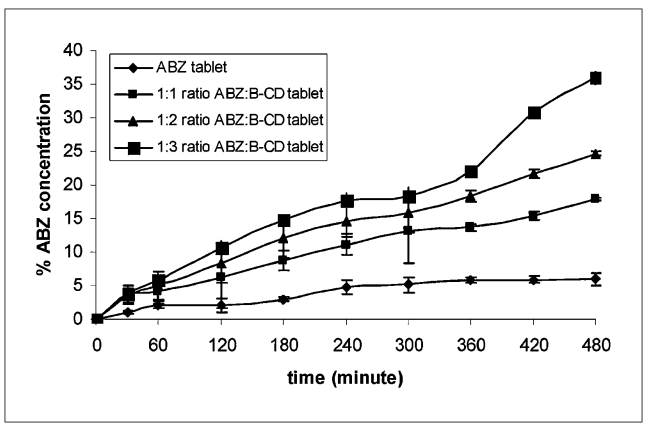

Fig. 6: Dissolution profiles of pure ABZ and ABZ: β-CD complex tablets at pH 7.4

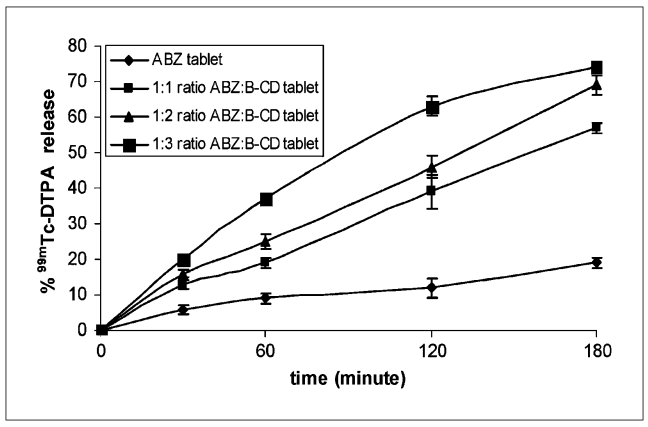

Fig. 7: Dissolution profiles of radiolabelled ABZ and ABZ:β-CD complex tablets at pH 1.2

in Figs. 7 and 8. In both media, the highest ^{99m}Tc-DTPA release was obtained from the complex containing the highest concentration of β-CD (1:3 w/w). In our study, the *in vitro* release results of radiolabelled tablets were compared with those of nonradiolabelled tablets. Similar dissolution profiles were obtained, while the best correlation was found obtained for the complex tablet with a 1:3 ABZ:β-CD ratio in pH 1.2 medium ($r^2 = 0.9923$).

2.7. Conclusions

This study was carried out to develop ABZ tablets based on ABZ:β-CD complexes in order to improve the solubility of ABZ. Complexes of ABZ with β-CD were prepared by a kneading method and characterized by NMR spectroscopy, SEM and FTIR spectroscopy. The kneading method is useful for obtaining true inclusion complexes. The phase solubility studies showed that drug solubility in pH 7.4 phosphate buffer solution improved with increasing β-CD concentration. The existence of a rising segment in the phase solubility diagram suggests that a soluble complex can be formed. Thus the dissolution of the drug can be enhanced by forming an inclusion complex. All the dissolution studies showed that ABZ:β-CD complex tablets dissolved faster than pure ABZ tablets. The amount of β-CD influenced the release of ABZ by changing the solubility of the drug. When the ratio of β-CD increased, the release of ABZ increased. A labelling procedure is often used to follow the release rates of drugs and vehicles (Szejtli 1988). For this purpose, ABZ tablets were wetted with ^{99m}Tc-DTPA and *in vitro* release rates were examined by gamma counting. According to these results, similar dissolution rates were found for radioactive and nonradioactive tablets. For this reason, in the future, we plan to conduct *in vivo* studies with ^{99m}Tc-DTPA/ABZ: β-CD complex tablets.

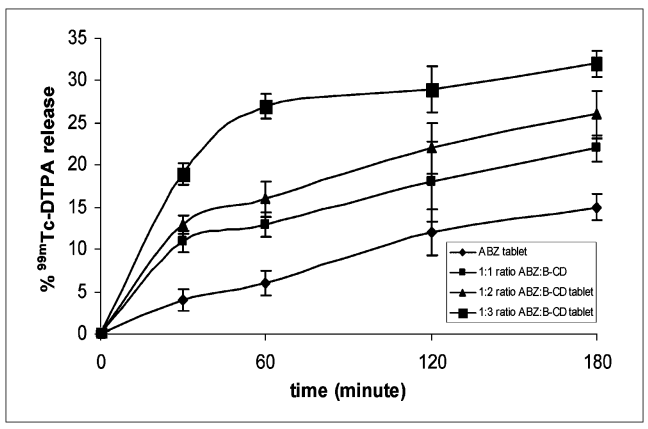

Fig. 8: Dissolution profiles of radiolabelled ABZ and ABZ:β-CD complex tablets at pH 7.4

3. Experimental

3.1. Materials

β-CD was supplied by Röhm GmbH (Darmstadt, Germany). ABZ was kindly provided by Biopharma Drug Company (Turkey). ^{99m}Tc-DTPA was supplied by the Department of Nuclear Medicine (Ege University, Turkey). All other chemicals and reagents were of analytical grade.

3.2. Preparation of ABZ:β-CD complex

The kneading method was used for preparation of the ABZ:β-CD complex. The following β-CD ratios were used: 1:1, 1:2 and 1:3 (w/w). The kneaded product was obtained by adding a mixture of methyl alcohol and distilled water (1:1 v/v) to the ABZ and β-CD powder mixture to obtain a homogeneous paste. This mixture was stirred for 30 min and dried at 37 °C for 30 min.

3.3. Phase solubility studies

The phase solubility studies were carried out at 37 °C in pH 7.4 buffer solutions. Different concentrations of CDs were used to prepare solutions in pH 7.4 buffer solution. An excess of ABZ was added to 20 mL of aqueous buffer solution containing increasing amounts of β-CD (ranging from 0 to 20 mM). The tubes were placed in a water bath at a constant temperature (± 25 °C), and were shaken with horizontal movements (100/min) for a period of time to allow equilibrium for 4 days. After these periods, the concentrations did not change. Then they were filtered (pore size 0.45 μm) and analyzed by UV spectrophotometry at 295 nm. Phase solubility curves were produced by plotting the total concentration of dissolved drug against the concentration of β-CD. The apparent stability constants (Ks) of the complexes were calculated from the slope of the phase solubility diagrams according to the following equation, where S_0 was the solubility of ABZ at 37 °C at each pH value in absence of β-CD (Rajendrakumar et al. 2005, Higuchi et al. 1965, Connors et al.1987).

$$Ks = \frac{slope}{So \times (1 - slope)}$$

3.4. In vitro characterization

3.4.1. FTIR spectroscopy

Samples were prepared by the potassium bromide disk method. Small amounts of each complex, ABZ and β-CD were sealed into potassium bromide pellets by a hydraulic press (200 kg/cm^2, 15 s). This pellet sample was directly inserted into the cell. The cell was then placed in the FTIR spectrometer (Perkin Elmer Spectrum100) and the procedure was carried out using a time–scan measurement program. Samples were scanned for absorbance from 4000 to 400 cm^{-1}.

3.4.2. ^{1}H NMR spectroscopy

Samples containing pure ABZ and ABZ:β-CD complexes were dissolved in DMSO (dimethylsulfoxide), β-CD was dissolved in D_2O and filtered before use. ^{1}H NMR spectra were obtained at 400 MHz and 25 °C. Chemical shifts are quoted relative to sodium 3-trimethylsilyl (D4) propionate at 0 ppm, but spectra were calibrated via the known position of the residual HOD resonance, which was used as a reference.

3.4.3. SEM analysis

Scanning electron images were obtained using a Philips XL-30 SEM. The instrument settings were as follows: 4-kV acceleration potential, 7–8 mm working distance, and condenser lens setting of 25. The samples were coated with about 10 nm of gold/palladium alloy using a Hummer 6.2 sputter coater. The magnification factor was adjusted so that a selected particle occupied the full field length, as follows: β-CD and ABZ product were magnified 600×, and 1:1, 1:2, 1:3 w/w ABZ:β-CD complex products were magnified 1000×.

3.5. Preparation of tablet formulations

The tablets were prepared by direct compression of the ABZ:β-CD complexes and ABZ with the excipients listed in Tables 1 and 2 using a powder fraction that had been passed through a 180-μm sieve. The powders were mixed and directly compressed using a Stokes F-press, model 900.519.2.

Table 1: Tablet formulations containing ABZ:β-CD complexes

Ingredient (%)	ABZ:β-CD (1:1)		ABZ:β-CD (1:2)		ABZ:β-CD (1:3)	
	ABZ	β-CD	ABZ	β-CD	ABZ	β-CD
ABZ: β-CD complex	10	10	10	20	10	30
Avicel	40		40		40	
Starch	40		30		20	

Table 2: Tablet formulations containing ABZ only

Ingredient (%)	Amount of powder
ABZ	10
Avicel	40
Starch	50

Table 3: Dissolution media for radiolabelled and nonradiolabelled tablets

Ratio of ABZ: β-CD complex	Radiolabelled tablet		Nonradiolabelled tablet	
1:1	pH 1.2	pH 7.4	PH 1.2	pH 7.4
1:2	pH 1.2	pH 7.4	PH 1.2	pH 7.4
1:3	pH 1.2	pH 7.4	PH 1.2	pH 7.4

For radiolabelling studies, ^{99m}Tc-DTPA was used as the marker to represent a soluble compound (Ofori-Kwakye et al. 2004). Tablets were labeled with ^{99m}Tc-DTPA during preparation. Then the tablets were dried for 15 min in an oven at 70 °C. In this way, each tablet adsorbed 0.1 mCi of ^{99m}Tc-DTPA.

3.6. Dissolution studies with tablets

Dissolution studies were carried out with both nonradiolabelled and radiolabelled tablets. The dissolution medium is shown in Table 3 for both tablets.

3.6.1. Dissolution studies with nonradiolabelled tablets

Dissolution studies of tablets were performed using USP apparatus 2, in 900 mL of 0.1 N HCl and pH 7.4 phosphate buffer, with a paddle speed of 50 rpm, at 37 °C. Each study was done in triplicate. Samples were withdrawn at scheduled time intervals (60, 120, 180, 240, 300, 360, 420 and 480 min). The amount of drug released was determined by UV spectrophotometry at 295 nm.

3.6.2. Dissolution studies with radiolabelled tablets

The radiolabelled tablets were placed in 900 ml of 0.1 N HCl (pH 1.2) and phosphate buffer (pH 7.4) at 37 °C, and the solutions were shaken at a spindle speed of 50 rpm. At appropriate time intervals, samples were withdrawn and replaced with fresh medium. The dissolution rate of the radiolabelled tablets was evaluated by counting in filtered samples with a gamma counter (SESA Uniscaler-I/S). All experiments were replicated at least three times.

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