Brazilian Journal
of Chemical
Engineering

ISSN 0104-6632
Printed in Brazil
www.abeq.org.br/bjche

Vol. 25, No. 02, pp. 255 - 267, April - June, 2008

ENHANCEMENT OF SOLUBILITY OF ALBENDAZOLE BY COMPLEXATION WITH β-CYCLODEXTRIN

C. Moriwaki[1], G. L. Costa[1], C. N. Ferracini[1], F. F. de Moraes[2], G. M. Zanin[2], E. A. G. Pineda[3] and G. Matioli[1*]

[1]Departamento de Farmácia e Farmacologia, Phone: + (55) (44) 3261-3868, Universidade Estadual de Maringá UEM, Av. Colombo 5790, Bloco P02, CEP: 87020-900, Maringá - PR, Brasil.
E-mail: gmatioli@uem.br
[2]Departamento de Engenharia Química, UEM, CEP 87020-900, Maringá - PR, Brasil.
[3]Departamento de Química, UEM, CEP 87020-900, Maringá - PR, Brasil.

(Received: August 17, 2006 ; Accepted: January 31, 2008)

Abstract - A high dosage of albendazole (ABZ) is required for treating systemic helminthe infections because of its low solubility. Aiming at increasing ABZ solubility, complexation with beta-cyclodextrin (β-CD) using aqueous and acetic acid solutions as ABZ solubilizer was studied. In aqueous β-CD, complexation increased solubility 53.4 times, giving a complex equilibrium constant of 1266 L mol^{-1} with a maximum ABZ concentration of 276 μmol L^{-1} for 16.3 mmol L^{-1} β-CD. For complexation in 1.05 mol L^{-1} acetic acid, UV absorbance spectra and ^{1}H-NMR analysis confirmed complex formation. The UV absorbance of ABZ/acid acetic/β-CD solutions was modeled by the formation of two complexes with molar ratios 1:1 and 1:2 ABZ:β-CD. When neutralized with NaOH these solutions did not form precipitates only for the ABZ:β-CD molar ratios of 1:8 and 1:10, showing that ABZ solubility could be increased 306 times. Results show that high β-CD molar ratios hold ABZ in solution by complexation enhanced by the acetate anion.
Keywords: Albendazole; Cyclodextrins; β-cyclodextrin; Complexation; Solubility.

INTRODUCTION

Albendazole (ABZ) is an antihelminthic drug derived from benzimidazole that has a broad spectrum of activity, good tolerance, and low cost. It has been used against human and animal helminthe parasites, such as nematodes, metacestodes, and hydatoses, for more than two decades (Gyurik et al., 1981; Evrard et al., 2002).

When used in lengthy therapies such as for hydatoses and neurocysticercosis, ABZ can produce gastrointestinal pain, severe headaches, fever, fatigue, hair loss, leukopenia, thrombocytopenia, and liver degeneration. Hence, it is not recommended for patients with hepatic problems. ABZ is teratogenic and embryotoxic and consequently cannot be administered to pregnant women. The safety of ABZ for children of less than two years has not yet been established (Hardman et al., 1996).

In some of the less developed regions of the world, helminthic intestinal infections are more common and they may impair ocular, muscle or blood systemic circulation (Evrard et al., 2002). The low cost and broad spectrum of activity of ABZ make it typically the drug of choice for these cases. However, its very low solubility results in low absorbance through the gastrointestinal tract and in some applications, such as for the systemic diseases, high oral doses that cause adverse gastrointestinal disturbances and several detrimental side effects are

*To whom correspondence should be addressed

required. Therefore, achieving greater ABZ solubility, for example through complexation with cyclodextrins (CDs), could increase bioavailability of the drug, which would be most advantageous in lengthy therapies (Bassani et al., 1996; Castillo et al., 1999; Garcia et al., 2003).

CDs are cyclic oligosaccharides composed of a variable number of glucose units (commonly 6 to 8), linked by α-1,4 bonds. They are produced by the reaction of starch with the cyclodextrin-glycosyltransferase enzyme (CGTase) (Matioli et al., 2000). The CD molecules are more hydrophilic externally and relatively hydrophobic in side their ring cavity. In liquid or occasionally solid media, CDs are able to form inclusion complexes with many different types of appropriately sized, preferentially nonpolar molecules (Frömming and Szejtli, 1994; Martin del Valle, 2004).

For some substances with low solubility, inclusion in CDs may improve their solubility and kinetics of dissolution. The active principle may cross the membranes with greater ease if the complex stability constant has intermediary values, and this translates into a higher in vivo bioavailability and simultaneous increase in therapeutical efficacy (Bekers et al., 1991). Solubility depends on the type of CDs. Increased solubility is obtained when the more commonly substituted CDs are used instead of nonderivatized CDs (Castillo et al., 1999). This occurs because highly soluble CD derivatives are used. Derivatization, such as that in hydroxypropyl-β-CD, increases aqueous solubility by imparting greater flexibility to the external CD hydroxyl groups, which also has the potential to increase hydrogen bond formation between the guest molecule and the derivatized CD and consequently may increase the stability of the inclusion complex. Several cyclodextrins have been considered for their ability to form an inclusion complex with ABZ. Of these cyclodextrin, 2-hydroxypropyl-β-CD (HP-β-CD) appears to be especially useful based on its safety for humans and its complexation potential (Evrard et al., 2002). However, the CD most commonly used for producing pharmaceutical formulations is β-CD, probably because it is the cheapest due to its low cost of production, since it is easily separated from the starch reaction mixture by crystallization (Martin del Valle, 2004; Szejtli, 1988).

A large number of pharmaceutical drugs have been encapsulated in β-CD aiming at improving their physicochemical proprieties (stability and solubility), bioavailability, and tolerance and reducing their adverse side effects (Frömming and Szejtli, 1994; Hamon and Moraes, 1990; Stella and Rajewski, 1997) some examples are acetyl salicylic acid, naproxen, piroxicam, ketoconazole, ibuprofen, ketoprofen, furosemide, tolbutamide, ABZ, etc (Szejtli, 1988).

Previous studies have already reported a synergistic effect of organic acids and CDs on the solubility of drugs, especially with citric acid. This type of solubility is generally attributed to a change in the solute-solvent interaction, such as ionization of guest molecules (Evrard et al., 2002).

This work was developed with the objective of studying the complexation of ABZ with β-CD in aqueous and acetic acid solutions as ABZ solubilizer to confirm complex formation and increase ABZ solubility with a nonderivatized CD. Both synergistic solubility enhancers, β-CD and acetic acid, were chosen because they are the cheapest and most readily available cyclodextrin and organic acid, allowing the production of a low-cost ABZ preparation intended for application in poorer regions of the world. It was also expected that, based on the lower molecular average pKa of acetic acid in comparison to that of citric acid, the guest molecule would be more ionized in the inclusion complex, increasing the solubilizing synergistic effect. The product of encapsulation was characterized by UV-V spectroscopy, infrared absorbance spectra, DSC, TGA, and NMR.

MATERIALS AND METHODS

Materials

ABZ (Smithkline Beecham Pharmaceutical, UK) and β-CD (Sigma Chemical Co., USA) were used. Other analytical grade reagents and distilled water were used throughout this work.

ABZ Specific Molar Absorbance

ABZ (5, 7.5, 10, 15, and 20 mg) was dissolved in 3 mL of concentrated acetic acid and water was added to give a volume of 50 mL. The solution absorbance was measured at 295 nm against an equivalent solution of acetic acid (1.05 mol L^{-1}) and a straight line was fitted to the data, yielding ABZ specific molar absorbance (ε_{ABZ}) as the slope.

ABZ:β-CD Phase Solubility Tests

ABZ and β-CD were mixed in the following molar ratios: 1:1, 1:2, 1:4, 1:6, 1:8, and 1:10 in accordance with a procedure similar to that given by Bassani et al. (1996). For the 1:1 molar ratio, ABZ (21.0 mg) was added to a solution of β-CD (92.5 mg in 50 mL water) and the suspension was kept under agitation (175 rpm) at 37 ºC for three days. Since β-CD has a limiting solubility of 18.5 g/L at 25 ºC, for all the ABZ:β-CD molar ratios, β-CD was in solution and the excess ABZ was in suspension. After the agitation period, the suspensions were cooled to 25ºC and the supernatants were filtered through a 0.45 μm Millipore membrane, diluted with water, and analyzed by UV spectroscopy as described in Characterization methods.

A similar protocol without β-CD was used for the direct determination of ABZ solubility in distilled water.

Study of Interaction Between ABZ and β-CD

The supernatant solutions from the phase solubility tests were evaporated until dry (rotaevaporator BUCHI, model RE 120) and solid samples were taken for characterization of the supposed ABZ:β-CD complex as indicated in Characterization methods.

Solubilization of ABZ Before Complexation

According to Szejtli (1988), a better complexation is achieved if the host and guest have previously been completely dissolved. Given the low solubility of ABZ in water, acetic acid was used as ABZ solvent for another series of complexation tests. In this case, 21 mg of ABZ was initially dissolved in concentrated acetic acid (3 mL) and then 47 mL of an aqueous solution of β-CD was added. The following molar ratios of ABZ to β-CD were used: 1:1, 1:2, 1:4, 1:6, 1:8, and 1:10. These liquid mixtures were analyzed by UV-VIS spectroscopy in accordance with Characterization methods.

Additionally, two physical mixtures of ABZ and β-CD were prepared at molar ratios of 1:1 and 1:8 and added to 3 mL concentrated acetic acid and water was added to give a total volume of 50 mL. These suspensions were kept under agitation for a short time (2 h at 37ºC) and then filtered.

Characterization Methods

a) UV-VIS spectroscopy and β-CD analyses

The ABZ:β-CD supernatants were characterized by UV-VIS spectroscopy using a Varian spectrophotometer, model Cary 50, at 200-400 nm, and noncomplexed β-CD was determined by colorimetry with the dye extinction method using phenolphthalein (PHE) (Hamon and Moraes, 1990). A solution of PHE was mixed with the ABZ:β-CD supernatants and the two guests molecules, ABZ and PHE, competed for complex formation with β-CD. The PHE/β-CD complex had negligible absorbance at 550 nm and the PHE solution was discolored proportionally to the PHE/β-CD complex formed; thus β-CD concentration can be inferred.

b) DSC, TGA, and FTIR Analyses

Thermal analyses are useful for determining whether the product of the complexation protocol is a true complex. Solid products were obtained by drying the filtered solutions from the complexation tests with molar ratios of 1:6, 1:8, and 1:10. Samples of 6 mg of these solids were analyzed by DSC and TGA using a Shimadzu calorimeter (DSC-50 and TGA-50) at a heating rate of 5°C/min in a nitrogen atmosphere (20 mL/min). Pure ABZ and β-CD were also analyzed for comparison.

The same solids and a sample of the 1:8 physical mixture were analyzed with a FTIR BOMEM spectrophotometer, model MB-100, with a resolution of 4 cm^{-1}. The spectrum measurements were performed with KBr disks in a frequency range of 4000 to 400 cm^{1}.

c) NMR Analysis

The solid product of complexation was also analyzed by NMR spectroscopy (H^{1}-NMR and C^{13}-NMR) with a Varian mercury plus BB model, run at 300 MHz. The samples were dissolved in deuterated dimethylsulfoxide (δ=2.49 ppm) and the conditions for the Fourier transform were an acquisition time of 3.641 s, a pulse angle of 45º, a retention time of 1.359 s, and a spectrum number of 32.

d) Neutralization of the Supernatants with NaOH

In another test, after the complexation procedure in which ABZ was first solubilized with acetic acid,

as described in Solubilization of ABZ before complexation, the filtered solutions were neutralized by the addition of solid NaOH and the solution was monitored for formation of precipitates. If the solution remained clear, it was a good indication that all the ABZ used (21 mg/50 mL) was held in solution owing to the formation of a complex with β-CD.

RESULTS AND DISCUSSION

ABZ Specific Molar Absorbance

Figure 1 shows the absorbance data at 295 nm for the ABZ acetic acid solutions, prepared as described in ABZ specific molar absorbance. The value calculated for the ABZ specific molar absorbance (ε_{ABZ}) was 7927.9 L mol^{-1} cm^{-1}.

ABZ:β-CD Phase Solubility Diagram

The phase solubility diagram, in which the total concentration of solubilized ABZ (C_{ABZ}) is plotted as a function of total β-CD concentration ($C_{\beta\text{-CD}}$), is shown in Figure 2. A straight line was fitted to the data and the ABZ:β-CD 1:1 equilibrium constant was determined in accordance with Higuchi and Connors (1965) by Equation (1):

$$K_{1:1} = \frac{s}{C_{ABZ,0}(1-s)} \quad (1)$$

where $C_{ABZ,0}$ is ABZ concentration in the absence of β-CD, obtained as the y-intercept, and s is the slope (Figure 2). The total concentration of solubilized ABZ (C_{ABZ}) was calculated as

$$C_{ABZ} = \frac{ABS_{pst}}{\varepsilon_{ABZ}} \quad (2)$$

where ABS_{pst} is the phase solubility test absorbance and ε_{ABZ} is the specific molar absorbance of ABZ at 295 nm. It is implicitly assumed in the Higuchi and Connors (1965) procedure that the ε_{ABZ} value does not change upon complexation with β-CD.

The calculated values for the equilibrium constant and ABZ concentration at zero β-CD concentration were $K_{1:1}$ = 1266 L mol^{-1} and $C_{ABZ,0}$ = 13.62 μmol L^{-1}, respectively. Since the maximum ABZ concentration obtained was 276 μmol L^{-1}, ABZ concentration was increased 20.3 times in relation to the $C_{ABZ,0}$ calculated value. Alternatively, the direct measurement of ABZ solubility in water gave 5.17 μmol L^{-1}, and using this value, the maximum ABZ concentration achieved with aqueous β-CD was 53.4 times greater.

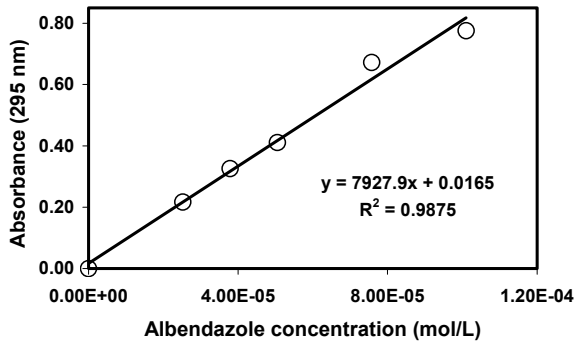


Figure 1: Determination of ABZ specific molar absorbance.

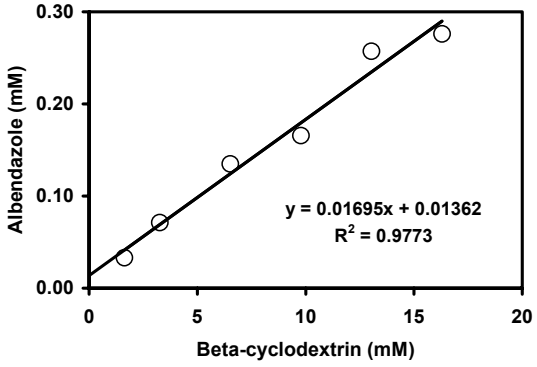


Figure 2: Phase solubility diagram of ABZ in aqueous β-CD.

Solubilization of ABZ Before Complexation

Solubilization of ABZ with acetic acid before complexation with β-CD allowed larger concentrations of ABZ in solution to be obtained. The UV spectrophotometric data obtained with the filtered ABZ/acetic acid/β-CD mixtures are shown in Figure 3.

In Figure 3 it can be observed that for 295 nm the absorbance peak increases with molar proportions of β-CD in the mixture for the smaller β-CD concentrations, indicating the formation of an ABZ:β-CD complex. At this wavelength, β-CD absorbance is negligible.

It is interesting to observe that the peak absorbance at 295 nm does not increase monotonically with higher β-CD concentrations, and this can be explained by the formation of two ABZ:β-CD complexes with molar ratios 1:1 and 1:2 ABZ:β-CD. The second complex should be prevalent for the higher β-CD concentrations and its absorbance per mol of ABZ should be smaller, so absorbance of an ABZ:β-CD mixture would have a local maximum as β-CD concentration increases (Figure 4). The curve drawn in Figure 4 results from a theoretical model based on equilibrium of the two complexes considered above (see Appendix).

The phenolphthalein assays of β-CD in the supernatant mixture indicated a lower concentration of free β-CD that in the same solution without ABZ. Therefore, this test also corroborated the formation of an ABZ:β-CD complex (Figure 5).

Fourier transform infrared spectroscopy did not show a significant change in position of the peaks at 1100 to 2000 cm^{-1}, as can be observed in Figures 6 and 7. Consequently, FTIR data did not allow the confirmation of complex formation. According to Frömming and Szejtli (1994), the characteristic FTIR bands associated with the inside of the CD are scarcely affected by the formation of a complex. These bands showed significant changes only when the mass of the guest molecule did not exceed 5 to 15% of the complex mass; otherwise the changes were masked by the guest spectrum. For a 1:1 ABZ:β-CD complex, ABZ corresponded to 18.9% of the complex mass, hence the negative result.

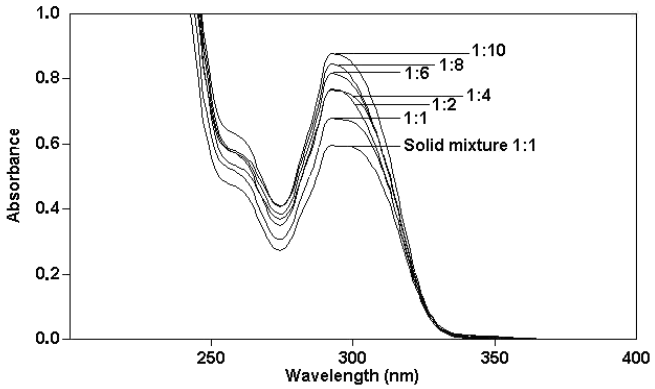


Figure 3: UV spectrum for the supernatant of ABZ/acetic acid/β-CD mixtures.

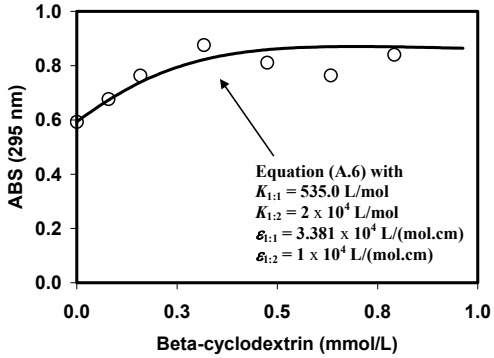


Figure 4: Absorbance at 295 nm for the filtered ABZ/acetic acid/β-CD mixtures.

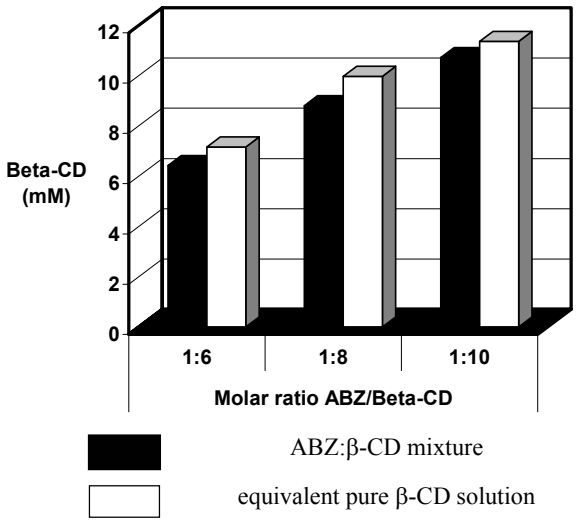


Figure 5: β-CD as given by the measurement with phenolphthalein.

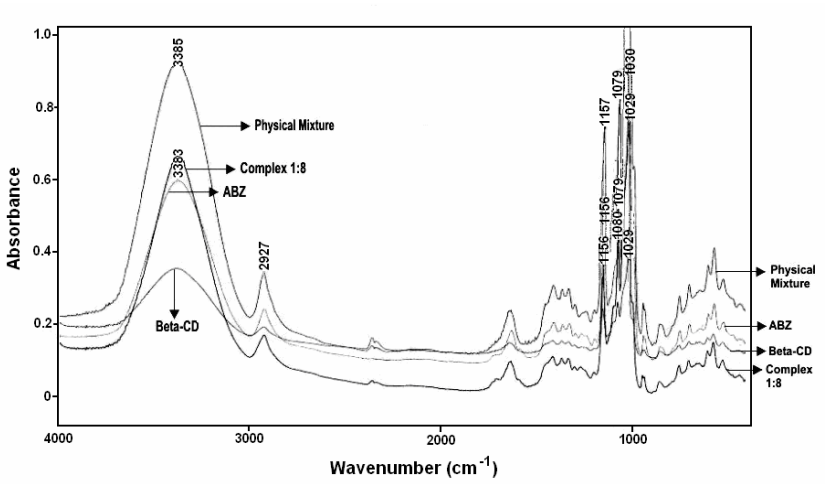


Figure 6: Infrared spectroscopy spectra in the range of 500 to 4000 cm^{-1}.

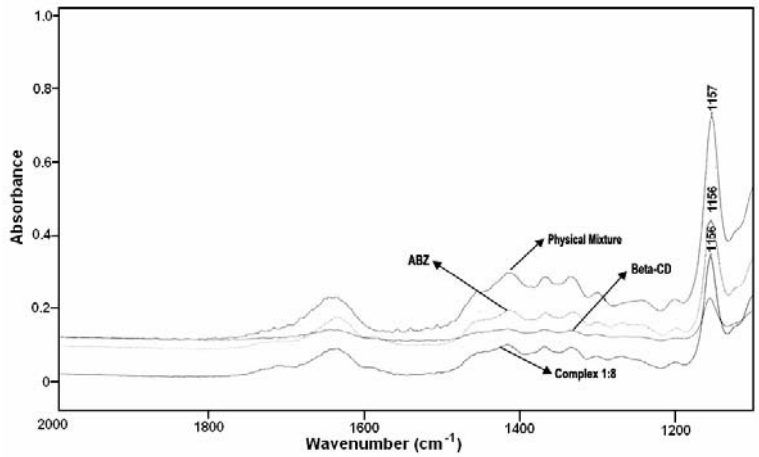


Figure 7: Infrared spectroscopy spectra in the range of 1100 to 2000 cm^{-1}.

Differential Scanning Calorimetry (DSC) and Thermogravimetry (TGA)

Thermal analyses are the first choice of analytical means for exact physicochemical characterization of the CD solid state in terms of energy required for water loss. Particular attention has been focused on the study of solid-vapor equilibrium for β-CD. In addition, the inclusion complexes formed with the CDs are usually prepared in an aqueous medium and can be considered a water-CD-guest molecule ternary system (Giordano et al., 2001).

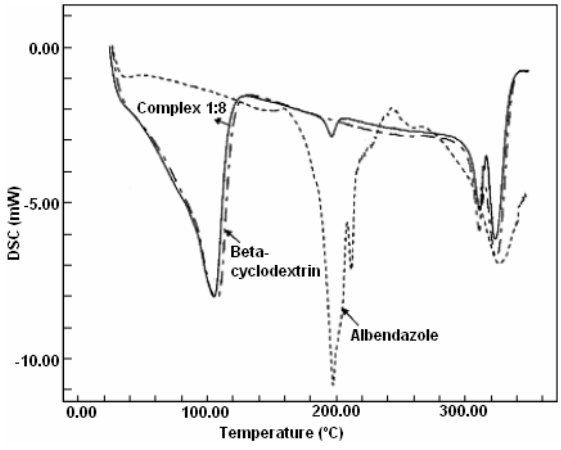
The inclusion complexes in which β-CD is the host can be subjected to thermal analysis if the melting or boiling points of the guest molecule are below the temperature of β-CD decomposition (250 to 300ºC) or if it is volatile in the range of 60 to 250ºC (Frömming and Szejtli, 1994).

In general, the occurrence of complexation is verified by the disappearance of the endothermic peak that is characteristic of the encapsulated molecule. This disappearance can be ascribed to an amorphous state, complex formation, or both (Szejtli, 1988). In our work, it was not possible to determine the melting point of the ABZ:β-CD complex, but rather a temperature range, 160 to 240ºC, as shown in Figure 8.a. According to Frömming and Szejtli (1994), the CDs do not have a definite melting point and above 200ºC they start to decompose. The observed thermoanalytical properties depend on at least four factors: water content, crystalline structure, heating rate, and gaseous environment. During TGA analysis, the CDs lost water below 100ºC and began to decompose above 250ºC. Therefore, the fact that a molecule does not melt at a given definite temperature impedes the confirmation of complex formation. In our work, this fact hindered verification of the ABZ:β-CD complex by DSC and TGA (Figure 8.c).

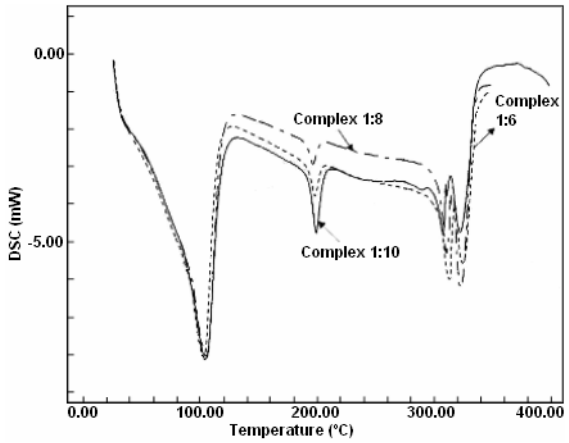
Castillo et al. (1999) analyzed pure ABZ by DSC and observed an endothermic peak at around 200.7ºC that corroborates the results in Figure 8.a. This peak, they observed, is not a simple peak, but in fact, can be considered a double peak that may be indicative of enantiomeric or polymorphic compounds. This factor also hinders the analysis of a possible ABZ:β-CD complex.

Thermal analysis of the complex preparations with molar ratios of 1:6, 1:8, and 1:10 (Figure 8.a and b) compared to the pure ABZ peak profile (Figure 8.a) indicates a reduction in the endothermic ABZ peak position at around 200ºC. However, it should be cautioned that this reduction might be considered the effect that in the complex preparations, ABZ is diluted by a much higher β-CD ratio. The peak profile in this case would then only reflect the weighed addition of the ABZ and β-CD peak thermograms.

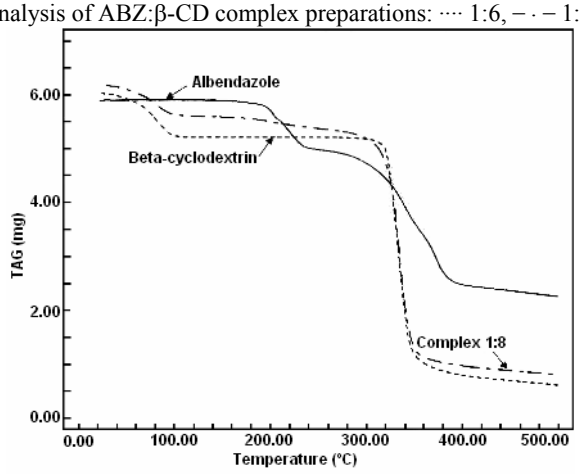
Castillo et al. (1999) also found these inconclusive results with DSC for the preparation of ABZ:hydroxypropyl-β-CD complex by coprecipitation, but they observed a true complex when the preparation method was freeze-drying.



(a) DSC analysis: ·····ABZ, – · – β-CD , — 1:8 Complex preparation



(b)DSC analysis of ABZ:β-CD complex preparations: ···· 1:6, – · – 1:8, — 1:10



(c) TGA analysis: ···· β-CD, – · – 1:8 Complex preparation, — ABZ

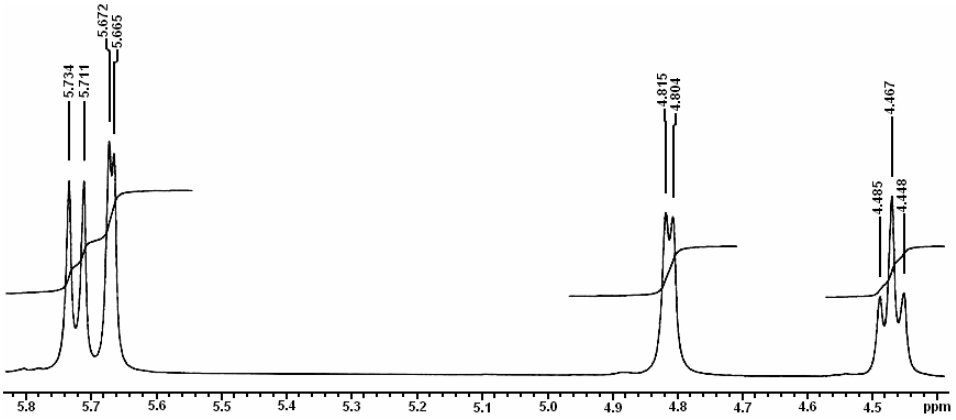
Figure 8: Thermal analyses of ABZ, CD, and their complex.

NMR Results

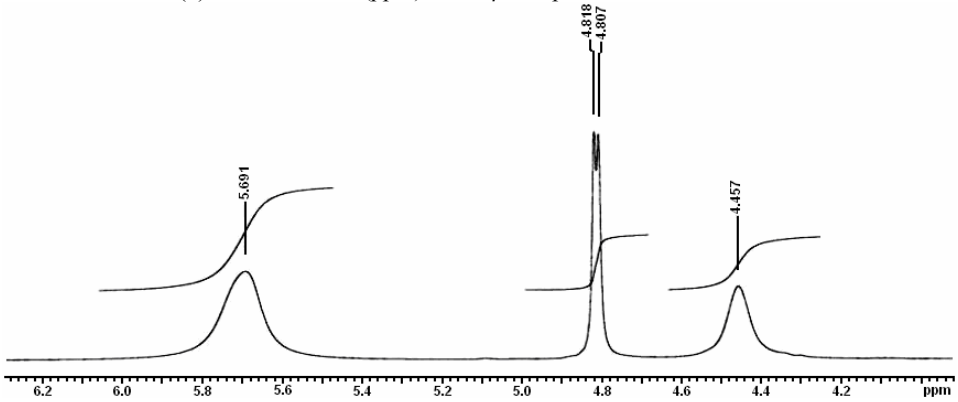
A variety of techniques are used to analyze complexes. Only nuclear magnetic resonance of protons (H^1-NMR) and carbon 13 (C^{13}-NMR) have proved the formation of a complex (Hedges, 1998). They are efficient for the identification and study of CD inclusion compounds, either in the solid or liquid state. β-CD has primary and secondary hydroxyls at the terminal regions of the CD toroidal structure. The H(3) and H(5) protons are directed towards the inside of the CD cavity, while H(1), H(2), and H(4) are found on the outside. Thus, when the guest molecule is lodged inside the cavity, H(3) and H(5), or close to it, H(6), have to be dislocated. Alternatively, when association occurs on the outside of the toroidal structure, H(1), H(2), and/or H(4) are necessarily affected (Szejtli, 1988).

Results from C^{13}-NMR analyses of the 1:8 complex preparation sample did not confirm the formation of the ABZ:β-CD complex (results not shown) because the chemical dislocations observed were insignificant. This result may be a consequence of the low molar ratio for ABZ and because aliphatic guests or included compounds with low association constants normally have negligible chemical dislocations (Wimmer et al., 2002).

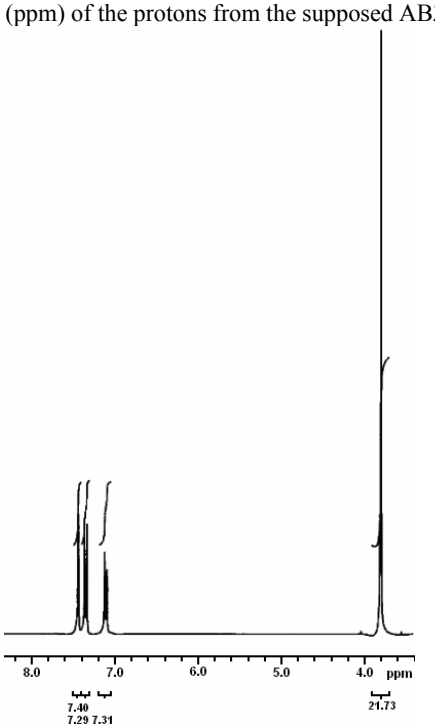
The H^1-NMR results obtained with the dried solids from the supernatant of the 1:8 ABZ:β-CD complex preparation are shown in Figure 9. Analysis of changes in the H^1 peak in the range of 5.6 to 5.8 ppm for the free ABZ and β-CD can be interpreted as a good indication of formation of the ABZ:β-CD complex because of the band broadening and change in peak intensity observed. Chemical dislocations (ppm) for the protons of the free β-CD and ABZ:β-CD complex (preparation molar ratio of 1:8) are shown in Table 1. β-CD proton H(1), present in the range of 5.6 to 5.8 ppm, had four chemical dislocations and proton H(5), seen in the range of 4.4 to 4.5 ppm, had three (see Figure 9.a also). The complex preparation had only one chemical dislocation (see Figure 9.b also), corroborating the inclusion of ABZ in β-CD.



(a) Chemical shift (ppm) of the β-CD protons in the free state



(b) Chemical shift (ppm) of the protons from the supposed ABZ:β-CD complex



(c) Chemical shift (ppm) of the ABZ protons in the free state

Figure 9: H^1-NMR results: (a) β-CD, (b) complex, (c) ABZ.

Table 1: Chemical dislocations (ppm) for the protons of free β-CD and the ABZ:β-CD complex (preparation molar ratio of 1:8).

Proton	H(1)	H(5)
Free β-CD	5.665	4.448
	5.672	4.467
	5.711	4.485
	5.734	
Complex	5.691	4.457

Neutralization of the Supernatants with NaOH

In Figure 10 it is shown that the filtered solutions of ABZ/acid acetic/β-CD mixtures neutralized with NaOH developed precipitates when the molar ratios were in the range of 1:1 to 1:4; the preparation with molar ratio 1:4 showed a fine precipitate at the surface, but for the higher molar ratios of 1:8 and 1:10, the solutions remained mostly clear. This indicates that for the molar ratios of 1:8 and 1:10, practically all the ABZ present was complexed with β-CD and the complex formed was soluble under these conditions. Therefore, the goal of increasing ABZ solubility with β-CD complexation was achieved, since the solution contained 21 mg of ABZ per 50 mL of solution. Using an ABZ solubility in water of 5.17 μmol L^{-1}, the maximum ABZ concentration achieved with 1.05 mol L^{-1} sodium acetate and 16.3 mmol L^{-1} β-CD was increased 306 times. The higher increase in ABZ solubility observed with the neutralized solutions indicates that the presence of acetate anion enhanced complexation by β-CD. Other authors have also obtained a synergistic effect for complexation with CDs in acidic media (Evrard et al., 2002; Castillo et al., 1999; Garcia et al., 2003).

Finally, it should be pointed out that enhancement of ABZ solubility has been studied by a number of authors (Evrard et al., 2002; Bassani et al., 1996; Castillo et al., 1999; Garcia et al., 2003), using preferentially hydroxypropyl-β-CD (HP-β-CD), which can increase ABZ solubility up to 3500 (Bassani et al., 1996) or even 10000 using citric acid as solubility enhancer (Evrard et al., 2002). However, ABZ is an inexpensive drug, frequently used for helminthiasis commonly found in poor or developing countries and because HP-β-CD is more expensive than β-CD, using HP-β-CD or HP-β-CD and citric acid raises the price of the medicine, thereby hindering commercialization.

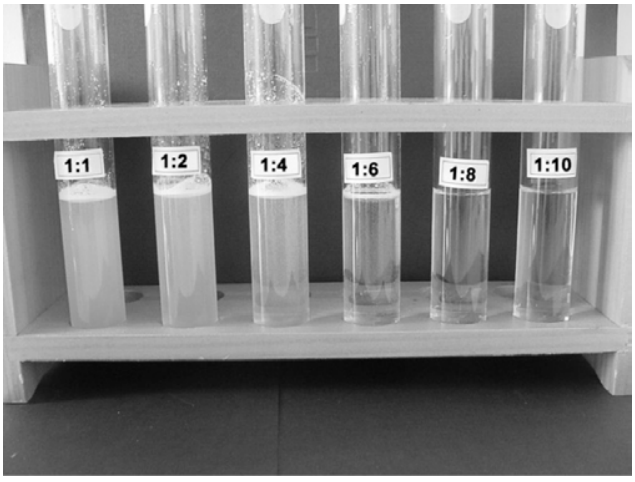


Figure 10: Neutralized ABZ/acid acetic/β-CD complex preparations (from left to right: ABZ:β-CD molar ratios of 1:1, 1:2, 1:4, 1:6, 1:8, and 1:10).

CONCLUSIONS

Spectrophotometric analyses of the preparations of ABZ:β-CD complex indicated the formation of a complex and the previous solubilization of ABZ with acetic acid allowed a more concentrated complex solution to be prepared. Still, infrared spectroscopy and thermal analyses by DSC and TGA did not confirm the formation of a complex, the latter because ABZ does not have a sharply defined melting point and has a polymorphous nature. C^{13}-NMR also failed to confirm the presence of a complex, but H^1-NMR yielded a good indication of complex formation because of band stretching and change in peak intensity observed for the complex, compared to those in the ABZ and β-CD standards. Neutralization of the filtered solutions of ABZ/acid acetic/β-CD mixtures allowed a very clear and visual result that was interpreted as a demonstration of complex formation.

The phase solubility diagram for the formation of the ABZ:β-CD complex in aqueous β-CD at 37ºC gave a straight line with an equilibrium constant of 1266 L mol^{-1} for the 1:1 molar ratio complex. The maximum ABZ concentration was 276 μmol L^{-1} in the presence of 16.3 mmol L^{-1} β-CD, resulting in a 53.4-time increase in ABZ concentration.

Complexation in the presence of acetic acid was modeled by the formation of two complexes with molar ratios 1:1 and 1:2 ABZ:β-CD, giving an adequate fit with equilibrium constants of 535.0 L mol^{-1} and 2 x 10^4 L mol^{-1}, respectively. When ABZ acetic acid solutions were mixed with β-CD they formed clear solutions that remained clear when neutralized with NaOH only for ABZ:β-CD ratios of 1:8 and 1:10. Precipitates formed with lower ABZ:β-CD ratios, suggesting that the mixtures with high β-CD molar ratios were more appropriate for ABZ complexation. In addition, a synergistic effect was observed for solubility enhanced by β-CD and the acetate anion. The 1:8 ABZ:β-CD molar ratio is preferred because it required less β-CD than the 1:10 case and provided the desired solubility effects. The goal of increasing ABZ solubility with β-CD complexation was achieved. ABZ solubility was increased up to 306 times in the presence of 1.05 mol L^{-1} sodium acetate and 16.3 mmol L^{-1} β-CD. Therefore, it was demonstrated that acetic acid in conjunction with β-CD is also a synergistic solubility enhancer pair of substances for ABZ and an alternative to more costly pairs such as hydroxypropyl-β-CD/citric acid already studied (Evrard et al., 2002).

Although, the β-CD/acetic acid pair showed a smaller potential for ABZ solubility enhancement than derivatized CD, such as HP-β-CD and citric acid, the lower cost of β-CD/acetic acid seems more appropriate for application in poorer regions of the world where improved ABZ medicine is most needed. Nonetheless, further studies are necessary to establish the pharmacological cost effectiveness and occasional side effects of the ABZ-β-CD/acetic acid preparation.

REFERENCES

Bassani, V. L., Krieger, D., Duchene, D. and Wouessidjewe, D., Enhanced water-solubility of albendazole by hydroxypropyl-beta-cyclodextrin complexation. Journal of Inclusion Phenomena and Macrocyclic Chemistry, 25, 149-152 (1996).

Bekers, O., Uijtendaal, E. V., Beijnen, J. H., Bult, A. and Underberg, W.J.M., Cyclodextrins in the pharmaceutical field. Drug Development and Industrial Pharmacy, 7, 1503-1549 (1991).

Castillo, J. A., Palomo-Canales, J., Garcia, J. J., Lastres, J. L., Bolas, F. and Torrado, J. J., Preparation and characterization of albendazole beta-cyclodextrin complexes. Drug Development and Industrial Pharmacy, 25, 1241-1248 (1999).

Evrard, B., Chiap, P., DeTullio, P., Ghalmi, F., Van Hees, T., Crommen, J., Losson, B. and Delattre, L., Oral bioavailability in sheep of albendazole from a suspension and from a solution containing hydroxypropyl-beta-cyclodextrin. Journal of Controlled Release, 85, 45-50 (2002).

Frömming, K. H. and Szejtli, J., Cyclodextrins in pharmacy. Kluwer Academic Publishers, Dordrecht (1994).

Garcia, J. J., Bolás, F. and Torrado, J. J., Bioavailability and efficacy characteristics of two different oral liquid formulations of albendazole. International Journal of Pharmaceutics, 250, 351-358 (2003).

Giordano, F., Novak, C. and Moyano, J.R., Thermal analysis of cyclodextrins and their inclusion compounds. Thermochimica Acta, 380, 123-151 (2001).

Gyurik, R. J., Chow, A.W., Zaber, B., Brumer, E. L., Miller, J. A., Petka, L. A. and Parish, R. C., Metabolism of albendazole in cattle, sheep, rats, and mice. Drug Metabolism and Disposition, 9, 503-508 (1981).

Hamon, V. and Moraes, F.F., Etude Preliminaire a L'immobilsation de L'enzyme CGTase WACKER. In Research Report. Laboratoire de Tecnologie Enzymatique, Compiègne, France: Université de

Tecnologie de Compiègne (1990).
Hardman, J. G., Gilman, A. G. and Limbert, L. E., Goodman & Gilman's The pharmacological basis of therapeutics. McGraw-Hill Companies, USA (1996).
Hedges, A. R., Industrial applications of cyclodextrins. Chemical Reviews, 98, 2035-2044 (1998).
Higuchi, T. and Connors, K. A., Phase solubility techniques. Advances in Analytical Chemistry and Instrumentation, 4, 117-212 (1965).
Martin del Valle, E. M., Cyclodextrin and their uses: A review. Process Biochemistry, 39, 1033-1046 (2004).
Matioli, G., Moraes, F.F. and Zanin, G.M., CDs e suas aplicações em: alimentos, fármacos, cosméticos, agricultura, biotecnologia, química analítica e produtos gerais. State University of Maringá Publisher (Eduem), Maringá (2000).
Stella, V. J. and Rajewski, R. A., Cyclodextrins: Their future in drug formulation and delivery. Pharmaceutical Research, 14, 556-567 (1997).
Szejtli, J., Cyclodextrin technology. Kluwer Academic Publishers, Dordrecht (1988).
Wimmer, R., Aachman, F., Larsen, K. L. and Petersen, S. B., NMR diffusion as a novel tool for measuring the association constant between cyclodextrin and guest molecules. Carbohydrate Research, 337, 841-849 (2002).

APPENDIX

ABZ:β-CD Complexation Model

Here a theoretical model for the complexation of ABZ with β-CD in the presence of an ABZ solvent, will be developed, considering the formation of two complexes with molar ratios of 1:1 and 1:2 ABZ:β-CD. The absorbance of the complex solution at 295 nm, ABS, measured against an equivalent solvent solution without ABZ and β-CD, is given by the sum of four contributions, namely

$$ABS = x\,\varepsilon_{ABZ} + y\,\varepsilon_{BCD} + z\,\varepsilon_{1:1} + u\,\varepsilon_{1:2} \qquad (A.1)$$

where x is the concentration of free ABZ, y is the concentration of free β-CD, z is the concentration of the 1 ABZ:1 β-CD complex, u is the concentration of the 1 ABZ:2 β-CD complex, ε_{ABZ} is the specific molar absorbance for free ABZ, $\varepsilon_{1:1}$ is the specific molar absorbance for the 1 ABZ:1 β-CD complex, $\varepsilon_{1:2}$ is the specific molar absorbance for the 1 ABZ:2 β-CD complex, and ε_{BCD} is the specific molar absorbance for free β-CD.

The two complexes are in thermodynamic equilibrium in accordance with the following reactions:

$$G + H \xrightleftharpoons{K_{1:1}} C, \quad K_{1:1} = \frac{z}{x\,y} \qquad (A.2)$$

(x) (y) (z)

$$C + H \xrightleftharpoons{K_{1:2}} D, \quad K_{1:2} = \frac{u}{z\,y} \qquad (A.3)$$

(z) (y) (u)

Stoichiometric relations for the species in solution:

$$x = a - z - u \qquad (A.4)$$

$$y = b - z - 2\,u \qquad (A.5)$$

where a is the total concentration of ABZ, and b is the total concentration of β-CD.

After some algebraic calculations, Equations (A.1) to (A.5) can be combined to yield

$$ABS = \frac{\left[\varepsilon_{ABZ} + K_{1:1}\,y\left(\varepsilon_{1:1} + K_{1:2}\,y\,\varepsilon_{1:2}\right)\right]\left(y - b - 2a\right)}{\left(2 + K_{1:1}\,y\right)} + y\varepsilon_{BCD} \qquad (A.6)$$

$$K_{1:1}\,K_{1:2}\,y^3 + K_{1:1}\left[K_{1:2}\left(2\,a - b\right) + 1\right]y^2 + \left[1 + K_{1:1}\left(a - b\right)\right]y - b = 0 \qquad (A.7)$$

The specific molar absorbance for free ABZ, ε_{ABZ}, can be calculated as

$$\varepsilon_{ABZ} = \frac{ABS_0}{a} \qquad (A.8)$$

where ABS_0 is the molar absorbance of the solution containing ABZ at concentration a and β-CD concentration is zero.

Given a, b, $K_{1:1}$, and $K_{1:2}$, y is first calculated as the real number solution of the cubic Equation (A.7). Then the complex solution absorbance is calculated with Equation (A.6), giving additionally, ε_{ABZ}, ε_{BCD}, $\varepsilon_{1:1}$, and $\varepsilon_{1:2}$. To reduce indetermination of the parameters, $K_{1:1}$ and $\varepsilon_{1:1}$ can be initially calculated with the points of low β-CD concentration and then these values are used with Equations (A.6) and (A.7) with $K_{1:2}$ and $\varepsilon_{1:2}$ still to be selected or determined by some procedure, such as minimization of the sum of the square of the errors for ABS, compared with the experimental data.