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Study of the interaction of β-cyclodextrin with albendazole in aqueous solutions

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ABSTRACT

We performed calorimetric titration (ITC) measurements of aqueous albendazole solutions with aqueous β-cyclodextrin solutions. The obtained results were used to determine the enthalpy and entropy of the drug–β-cyclodextrin interaction, the stoichiometry of the resulting inclusion complex and its formation constant. Using the UV spectrophotometer, we determined the solubility of albendazole in water, as well as the increase of the drug solubility driven by the increase of β-cyclodextrin concentration. Biological studies carried out on mouse cultures confirmed an increase of the albendazole–β-cyclodextrin complex bioavailability, as compared to the uncomplexed drug.

1. Introduction

Albendazole (methyl *N*-(6-propylsulfanyl-1H-benzimidazol-2-yl) [1,2] is a crystalline substance (Fig. 1), practically insoluble in water [3–5].

Albendazole (ABZ) is used as a broad-spectrum anthelmintic drug [6,7], especially in human and veterinary medicine. The effect of the drug is to inhibit the tubulin polymerization process. This results in an impaired glucose uptake leading to the depletion of glycogen reserve, which in turn reduces the amount of ATP produced in the body of the parasite, causing its death [8–10]. This compound exhibits bioactivity against certain parasites, both human and animal [11]. Albendazole also exerts anti-tumor effects [12–15]. This substance is characterized by low bioavailability [16]. Due to the poor absorption of albendazole by the gastrointestinal tract (<5%), it is most often administered as a suspension [17], microspheres [18] or nanoparticles [19].

Considering the low solubility of albendazole in water, it is interesting to study interactions of this drug with natural cyclodextrins. The characteristic structure of cyclodextrins enables the inclusion of poorly soluble, non-polar organic molecules inside the hydrophobic cavity. The formation of the inclusion complex not only increases the solubility of hydrophobic drugs in water [20], but also contributes to the masking of their unpleasant taste or odor [21,22]. Drug molecules included within cyclodextrin are gradually released into body fluids [23].

The aim of the current study was to determine the formation constant of the albendazole-β-cyclodextrin complex, to establish the number of hydrophobic drug molecules included within the β-cyclodextrin cavity, and to determine the physicochemical parameters of the resulting complex. These objectives were achieved using isothermal titration calorimeter (ITC). Furthermore, studies of the increase of albendazole solubility in water due to the increase of β-cyclodextrin concentration were also performed. Finally, the bioavailability of albendazole included within β-cyclodextrin has also been studied in mouse cultures, confirming the increase of bioavailability of the formed complex, as compared to the pure drug.

2. Materials and methods

2.1. Materials

Albendazole (99%) and β-cyclodextrin (98%) (all Sigma-Aldrich) were dried under vacuum in a Binder VD23 vacuum oven at 373.15 K for about 72 h. Aqueous solutions of the tested drug were prepared using analytical balance (Mettler Toledo AE 240), accurate to 1×10^{-5} g. Water used for calorimetric and UV spectrophotometric measurements was triple distilled, deionized and degassed.

2.2. Methods

2.2.1. UV–VIS spectrophotometry

Spectrophotometric measurements were carried out using a single-beam spectrophotometer SPECORD 50 (Analytik Jena). Measurements were performed in a 1 cm quartz cuvette.

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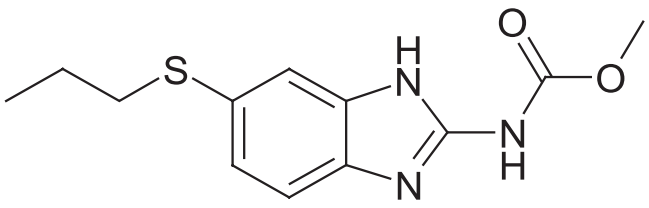

Fig. 1. Chemical structure of albendazole (ABZ).

Calibration curve for aqueous solutions of albendazole (ABZ) at concentrations from $4.13 \cdot 10^{-7}$ to $1.6 \cdot 10^{-5}$ mol (ABZ)/dm^3 (H_2O) was generated at the analytical wavelength $\lambda_{max} = 296$ nm. The determined dependence of albendazole absorbance against the drug concentration in water (Fig. 2) is described by the equation $y = 7602.3x$, ($R^2 = 0.9975$). The calculated molar extinction coefficient for albendazole at the analytical wavelength $\lambda_{max} = 296$ nm is $\varepsilon_o = 7602\ M^{-1} \cdot cm^{-1}$. This value is consistent with the literature value $\varepsilon_o = 7927$ [24].

In order to determine the albendazole solubility in water and the increase of its solubility in aqueous β-cyclodextrin solutions, a series of aqueous solutions of cyclic oligosaccharide (β-CD) with increasing concentrations of 0.5 mM (β-CD) to 13 mM (β-CD) were prepared. An excess of solid albendazole (ABZ) was introduced into Eppendorf tubes filled with water and twelve aqueous solutions of β-cyclodextrin. The solutions were thermostated for 7 days in an ultrasonic bath to reach the state of equilibrium. After 7 days a clear supernatant was collected and diluted with water so that the recorded absorbance values were within the spectrophotometer operating range. For each tested concentration of β-cyclodextrin six independent measurements were performed.

2.2.2. Isothermal titration calorimetry (ITC)

Calorimetry measurements were carried out using a VP-ITC isothermal microcalorimeter (MicroCal, USA). All titrations were performed at 298.15 K. The sample cell of 1.4275 μl was filled with the 0.072 mM aqueous solution of albendazole (ligand). A 287 μl syringe contained a 1.4 mM aqueous solution of β-cyclodextrin (receptor). Titrant solutions were injected from the syringe in 60 portions, 4 μl each. The duration time of the injection was 8 s, the injections were made at intervals of 400 s, at an agitator (syringe) rotational speed of 307 rpm.

Dilution measurements of aqueous solutions of β-cyclodextrin in water and the dilution measurements of aqueous solutions of antiparasitic drug with water were also performed. The measuring procedure was identical for all three measuring series (Fig. 3).

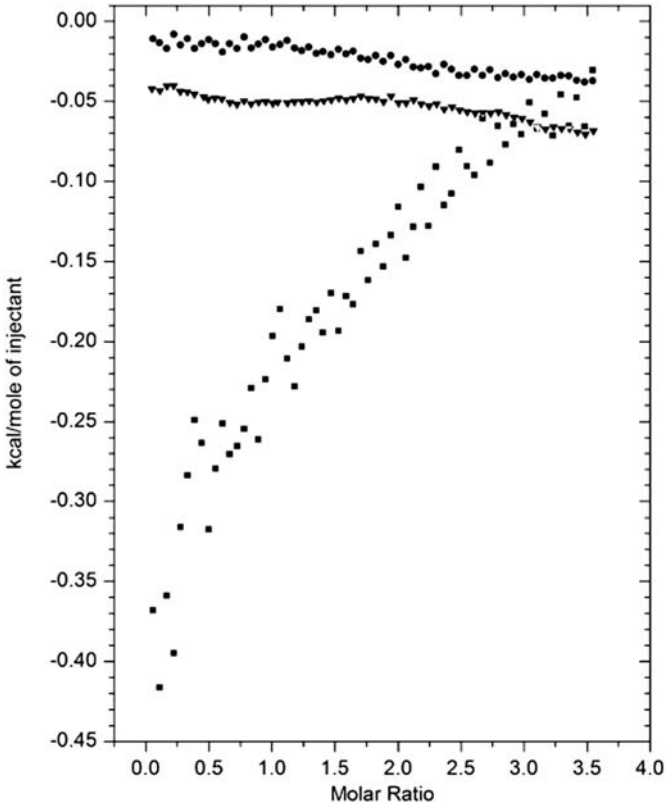

Fig. 3. Thermograms describing energetic effects during: the titration of aqueous 0.072 mM solution of albendazole with aqueous 1.4 mM solution of β-cyklodekstryny (■), dilution of aqueous 1.4 mM solution of β-cyclodextrin in water (▼) and dilution of 0.072 mM aqueous solution of albendazole with water (●).

2.2.3. Biological studies on mice

In order to assess the bioavailability of albendazole (ABZ) included within β-cyclodextrin (βCD) an experiment was performed on a group of 50 mice weighing 20 to 22 g from the lines C57BL/6. Animals were divided into 5 groups of 10 mice:

1st - control group received basic food and water *ad libitum*; 2nd - received with food Albendavet (10% albendazole powder) at a dose of 5000 mg (ABZ)/kg (mice body weight) of active substance; 3rd - received with food Albendavet at a dose of 12,000 mg (ABZ)/kg (mice) of active substance; 4th - received with food albendazole with β-cyclodextrin at a dose of 5000 mg (ABZ)/kg (mice) of active substance;

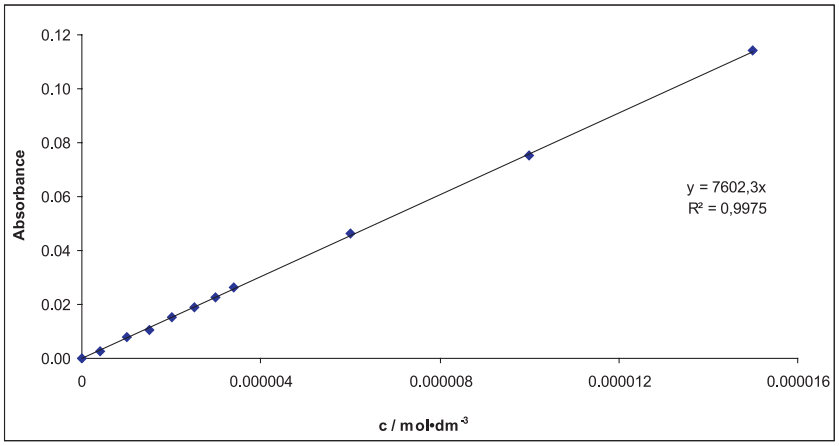

Fig. 2. Calibration curve for aqueous solution of albendazole at wavelength λ = 296 nm.

Table 1
Concentration and absorbance of albendazole (ABZ) in water under the influence of increased content of β-cyclodextrin (β-CD).

$C_{\beta CD}$/mol·dm^{-3}	A	C_{ABZ}/mol·dm^{-3}
0.013	0.3704	0.000974
0.012	0.3164	0.000832
0.011	0.3090	0.000827
0.010	0.2908	0.000765
0.009	0.2863	0.000705
0.008	0.2832	0.000693
0.006	0.2752	0.000565
0.005	0.2534	0.000438
0.004	0.2182	0.000427
0.003	0.1845	0.000328
0.002	0.0958	0.000252
0.001	0.0789	0.000179
0.000	0.0629	0.000083

5th - received with food albendazole with β-cyclodextrin at a dose of 12,000 mg (ABZ)/kg (mice) of active substance.

Complex of albendazole with β-cyclodextrin administered to mice was obtained as a result of grading moist mixture of cyclodextrin with albendazole in respect of stoichiometry equivalent equal 1(ABZ):1(βCD).

3. Results and discussion

3.1. UV–VIS spectrophotometry

The determined molar extinction coefficient of albendazole (ε_o = 7602 $M^{-1}\cdot cm^{-1}$) was used to establish the maximum solubility of albendazole (ABZ) in water. A clear supernatant of dissolved drug collected above the undissolved albendazole showed a concentration of 0.083 mmol (ABZ)/dm^3 (H_2O). This data is consistent with the literature values [25,26].

In order to determine the solubility increase of the hydrophobic drug (ABZ) in water, in the presence of β-cyclodextrin, the concentration of dissolved albendazole in aqueous solutions with β-cyclodextrin was determined spectroscopically. The calculated average absorbance values of albendazole in aqueous solutions of β-cyclodextrin with increasing concentration are shown in Table 1.

The dependence of albendazole concentration as a function of β-cyclodextrin concentration (Fig. 4) is described by the linear equation y = 0.0652x ± 0.000083 (R^2 = 0.9953), indicating a proportional increase of the drug solubility due to the increase of cyclodextrin concentration. The maximum solubility of albendazole in aqueous solution was calculated from the above equation, showing a level of 0.974 mmol

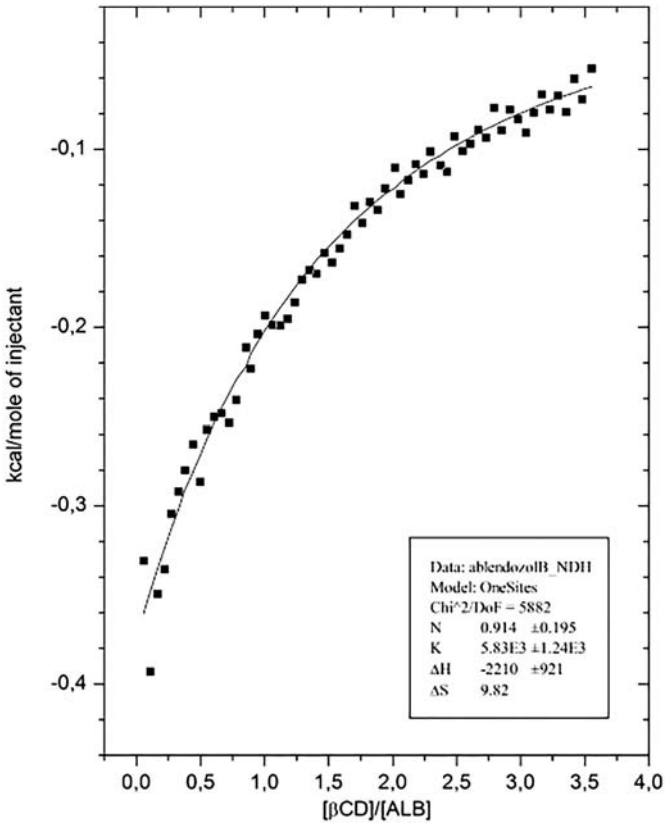

Fig. 5. Energetic effects of interaction between albendazole (ABZ) and β-cyclodextrin (β-CD) in water.

(ABZ)/dm^3 (H_20), at the maximum concentration of β-cyclodextrin in water (13 mmol β-CD/dm^3 H_2O).

The linear dependence of the albendazole concentration increase, due to the increase of β-cyclodextrin concentration (Fig. 4), suggests a 1:1 stoichiometry of the resulting complex [27]. Thus, the equation proposed Higuchi and Connors [28] was used to determine the formation constant value of the albendazole β-cyclodextrin complex [27].

$$K_{UV} = \frac{\text{slope}}{S_0(1-\text{slope})} \quad (1)$$

where: S_0 - determined solubility of albendazole in water (0.083 mmol ABZ/dm^3 H_2O),

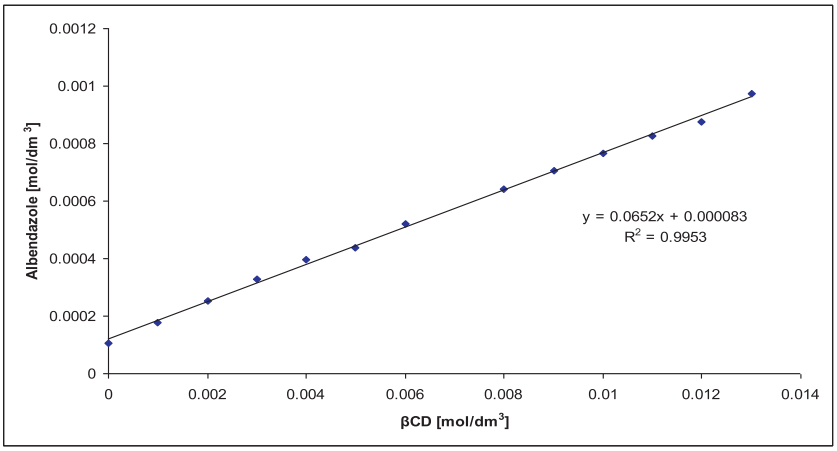

Fig. 4. Dependence of albendazole solubility on the concentration of β-cyclodextrin (β-CD) in aqueous solutions.

Table 2
Thermodynamical parameters of complex formation between albendazole and β-cyclodextrin in water.

	H_2O
N	0.914 ± 0.195
K	$5.83 \times 10^3 \pm 1.24 \times 10^3$ [M^{-1}]
ΔH	-2210 ± 921 [cal mol^{-1}]
ΔS	9.82 [cal mol^{-1} K^{-1}]
ΔG	-5147 [cal mol^{-1}]
K_{UV}	842 [M^{-1}]

The determined value of the formation constant for the albendazol–β-cyclodextrin complex is $K_{UV} = 8.42 \times 10^2$ M^{-1}. This value is consistent with the literature value [24].

3.2. Isothermal titration calorimetry (ITC)

The thermal effect describing a direct interaction of albendazole (ligand) with β-cyclodextrin (receptor) (Fig. 5) was obtained by subtracting the thermal effects accompanying the dilution of the aqueous solution of β-cyclodextrin in water and dilution of aqueous albendazole solutions with water from the thermal effect of the titration of the aqueous albendazole solution with aqueous β-cyclodextrin solution.

The direct thermal effects of albendazole interaction with β-cyclodextrin as a function of the composition of the titrated solution (Fig. 5) are described with the One set of sites model. The following thermodynamic parameters were determined: molar enthalpy (ΔH) and entropy (ΔS) of the polysaccharide (β-CD) – drug (ABZ) interaction, mean number of β-cyclodextrin molecules including hydrophobic fragments of albendazole molecules (N) and the formation constant of the antiparasitic drug – cyclodextrin complex. The values of thermodynamic parameters describing interactions between the tested substances in water are presented in Table 2.

The mean stoichiometric ratio (N) determining the number of β-cyclodextrin macromolecules including one albendazole molecule is within the measurement uncertainty limits and close to unity $N = 0.914 \pm 0.195$ (Table 2). This indicates the formation of the type 1 inclusion complex 1 βCD:1 ABZ. The formation of this stable complex with 1:1 stoichiometry between cyclodextrin and albendazole has been repeatedly confirmed in the literature by the ^{1}H NMR [29–31] and molecular modeling [32] methods.

The value of the constant ($K = 5.83 \times 10^3$ M^{-1}) (Table 2) characterizing the interactions between β-cyclodextrin (βCD) and albendazole (ALB) in water indicates the formation of a stable receptor-ligand inclusion complex. The value of the complex formation constant calculated by the ITC method significantly deviates from the value determined by the solvent method of Higuchi and Connors ($K_{UV} = 842$ M^{-1}). The discrepancies in the formation constant values most probably result from the fact that the UV measurements were preformed statically, while the ITC measurements dynamically. Differences in the formation constant values obtained by dynamic and static methods are confirmed by previous studies [33,34]. The determined change of the molar enthalpy value of the interaction between β-cyclodextrin and albendazole is negative (ΔH < 0) (Table 2), indicating an energetically favourable ligand-receptor interaction. The change in molar entropy of the investigated process has a positive value (ΔS > 0) (Table 2), indicating the spontaneous nature of the inclusion process. Also, the value of the free enthalpy change (ΔG < 0) (Table 2), calculated based on the obtained thermodynamic functions, confirms the spontaneity of the inclusion of a nonpolar ligand molecule into the hydrophobic receptor cavity (β-CD–ABZ).

Table 3
Mice survivability after toxic albendazole (ABZ) dose and albendazole–β-cyclodextrin complex.

	Dose mg (albendazole)/kg (mice body weight)	
	5000 mg (ABZ)/kg (mice)	12,000 mg (ABZ)/kg (mice)
Control group	10/10[a] (0)[b]	
Albendavet (ABZ)	10/10[a] (0)[b]	10/10[a] (0)[b]
Albendazole + βCD	10/10[a] (0)[b]	10/7[a] (30)[b]

[a] Total number of mice/mice that survived (index of mortality).
[b] Mice mortality (%).

3.3. Biological studies on mice

A mortality rate study was performed in mice receiving albendazole (ABZ) in free and complexed form (Table 3).

The study showed that the dose of 5000 mg (ABZ)/kg (mice body weight) did not cause mice death (Table 3). The number of mice that survived (index of mortality %) in respect of a toxic dose of albendazole equal 12,000 mg (ABZ)/kg (mice) was 0% it means 10/10 (total number of mice/mice that survived), while in the case of the complex of albendazole – cyclodextrin (12,000 mg/kg) mice mortality was 30%, 10/7 (Table 3).The obtained results (Table 3) indicate an increase in the bioavailability of complexed albendazole, as compared to the uncomplexed drug. Albendazole is classified as moderately toxic due to poor membrane permeability (5%) [35,36]. The formation of a complex with β-cyclodextrin causes an increase in its solubility more than ten times and results in a much easier permeation through the cell membrane. As a result, the toxic effect of the drug is clearly increasing (mice mortality).

4. Conclusion

UV spectrophotometric studies confirm the increase of water solubility of albendazole due to the increasing cyclic oligosaccharide (β-CD) concentrations. The experimentally determined water solubility of the investigated antiparasitic drug included within the β-cyclodextrin cavity increases approximately 12–fold. The ITC calorimetric titrations of aqueous solutions of albendazole with aqueous solution of β-cyclodextrin (ΔH < 0, ΔS > 0 and ΔG < 0, see Table 2) suggest spontaneous formation of a stable inclusion complex of antiparasitic drug (ABZ) β-cyclodextrin (β-CD) with a stoichiometry of 1(β-CD): 1(ABZ), whose composition is also confirmed by the UV spectroscopic studies. Using the ITC method and the measurements of solubility increase, values of the complex formation constants (Table 2) were determined, which indicate the formation of a stable β-cyclodextrin complex with albendazole. Biological studies of the albendazole–β-cyclodextrin complex in mice showed an increase in bioavailability of albendazole included within the β-cyclodextrin macromolecule, as compared to the uncomplexed drug.

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