

# Computational analysis and physico-chemical characterization of an inclusion compound between praziquantel and methyl- $\beta$ -cyclodextrin for use as an alternative in the treatment of schistosomiasis

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**Abstract** Schistosomiasis is still an endemic disease in many regions, with 250 million people infected with *Schistosoma* and about 500,000 deaths per year. Praziquantel (PZQ) is the drug of choice for schistosomiasis treatment, however it is classified as Class II in the Biopharmaceutics Classification System, as its low solubility hinders its performance in biological systems. The use of cyclodextrins is a useful tool to increase the solubility and bioavailability of drugs. The aim of this work was to prepare an inclusion compound of PZQ and methyl- $\beta$ -cyclodextrin (MeCD), perform its physico-chemical characterization, and explore its *in vitro* cytotoxicity. SEM showed a change of the morphological characteristics of PZQ:MeCD crystals, and IR data supported this finding, with changes after interaction with MeCD including effects on the C–H of the aromatic ring, observed at  $758\text{ cm}^{-1}$ . Differential scanning calorimetry measurements revealed that complexation occurred in a 1:1 molar ratio, as evidenced by the lack of a PZQ transition temperature after inclusion into the MeCD cavity. In

solution, the PZQ UV spectrum profile in the presence of MeCD was comparable to the PZQ spectrum in a hydrophobic solvent. Phase solubility diagrams showed that there was a 5.5-fold increase in PZQ solubility, and were indicative of a type  $A_L$  isotherm, that was used to determine an association constant ( $K_a$ ) of  $140.8\text{ M}^{-1}$ . No cytotoxicity of the PZQ:MeCD inclusion compound was observed in tests using 3T3 cells. The results suggest that the association of PZQ with MeCD could be a good alternative for the treatment of schistosomiasis.

**Keywords** Methyl- $\beta$ -cyclodextrin · Inclusion complexes · Schistosomiasis · Praziquantel

## Introduction

Schistosomiasis is still endemic in many regions, with 250 million people infected with *Schistosoma* and about 500,000 deaths per year. Praziquantel (PZQ) is a broad-spectrum drug used for the treatment of different helminthiases (Fig. 1a). However, experiments conducted in three different laboratories (in Italy, Egypt and England) have provided disparate results concerning PZQ application. Problems of resistance and reduced susceptibility are well described in the literature [1–3], and treatment failures could indicate that the schistosome is evolving towards future resistance, as has been experienced with other drugs, such as hycanthone [4]. At present, resistance of the parasite is not an important public health concern, however it could cause complications in schistosomiasis treatment in the future [2, 5].

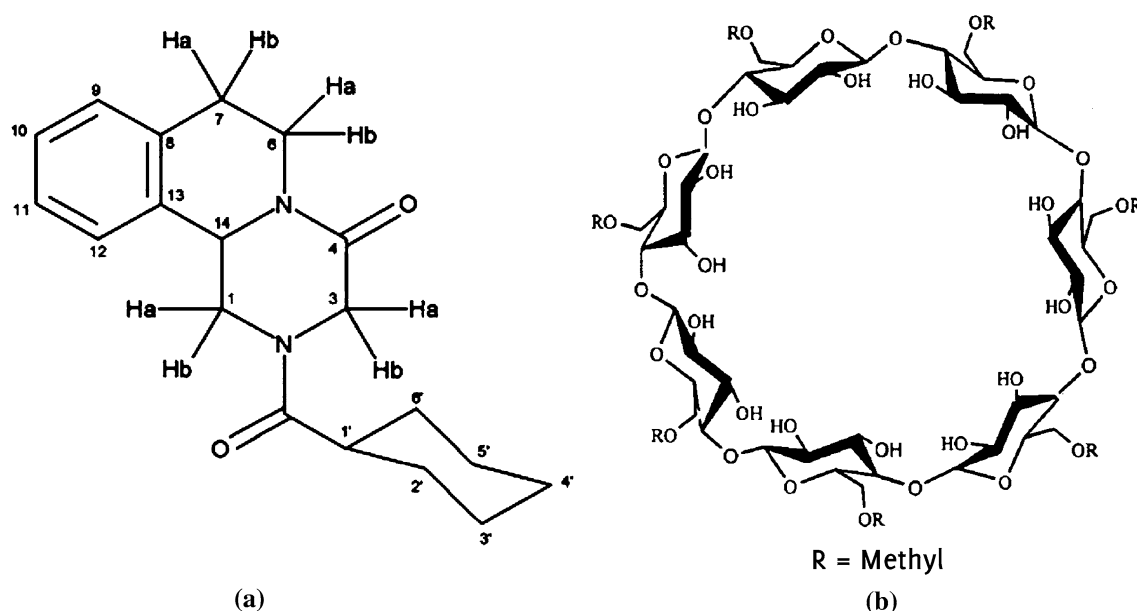
The physico-chemical properties of PZQ include high permeability; however, its solubility is extremely low, so that PZQ is classified as a Class II drug in the Biopharmaceutics

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**Fig. 1** Molecular structures of **a** praziquantel and **b** methyl- $\beta$ -cyclodextrin

Classification System (BCS) [6]. This limitation means that it is less available in the organism [1, 3, 7].

Cyclodextrins (CDs) have been used in various commercially successful pharmaceutical products, increasing solubility and improving drug bioavailability [8]. These compounds are cyclic polysaccharides, formed by the connection of glycosidic units in a cone-like shape. The natural  $\alpha$ ,  $\beta$  and  $\gamma$ -CDs possess 6, 7 and 8 units of glucose, respectively. The presence of hydroxyl groups gives them a hydrophilic exterior, and a hydrophobic cavity able to include molecules, hence changing their physico-chemical characteristics [8].

Natural CDs, especially  $\beta$ -CD, have limited aqueous solubility that could lead to precipitation of the solid complexes formed. However, changes in the saccharide can be used to modify the CDs and increase solubility. Methyl- $\beta$ -cyclodextrin (MeCD) is a modified CD with a methoxy group instead of primary hydroxyls [9, 10] (Fig. 1b). It has an increased aqueous solubility (approximately 20 times greater), and different to  $\beta$ -CD, which is only safe for oral administration, MeCD is suitable for both oral and parenteral routes [11, 12].

A previous paper [13] showed that PZQ could form an inclusion complex with  $\beta$ -CD, and subsequently there have been reports that the incorporation of PZQ into cyclodextrins provides better performance of the drug, increasing its bioavailability during schistosomiasis treatment [14–16]. The approach of this work was to prepare and characterize inclusion compounds using methyl- $\beta$ -cyclodextrin and PZQ. Improvement of the solubility profile of PZQ, and consequently its bioavailability and therapeutic efficacy, could provide an alternative route for the administration of this medicine.

## Experimental and theoretical

### Computational analysis

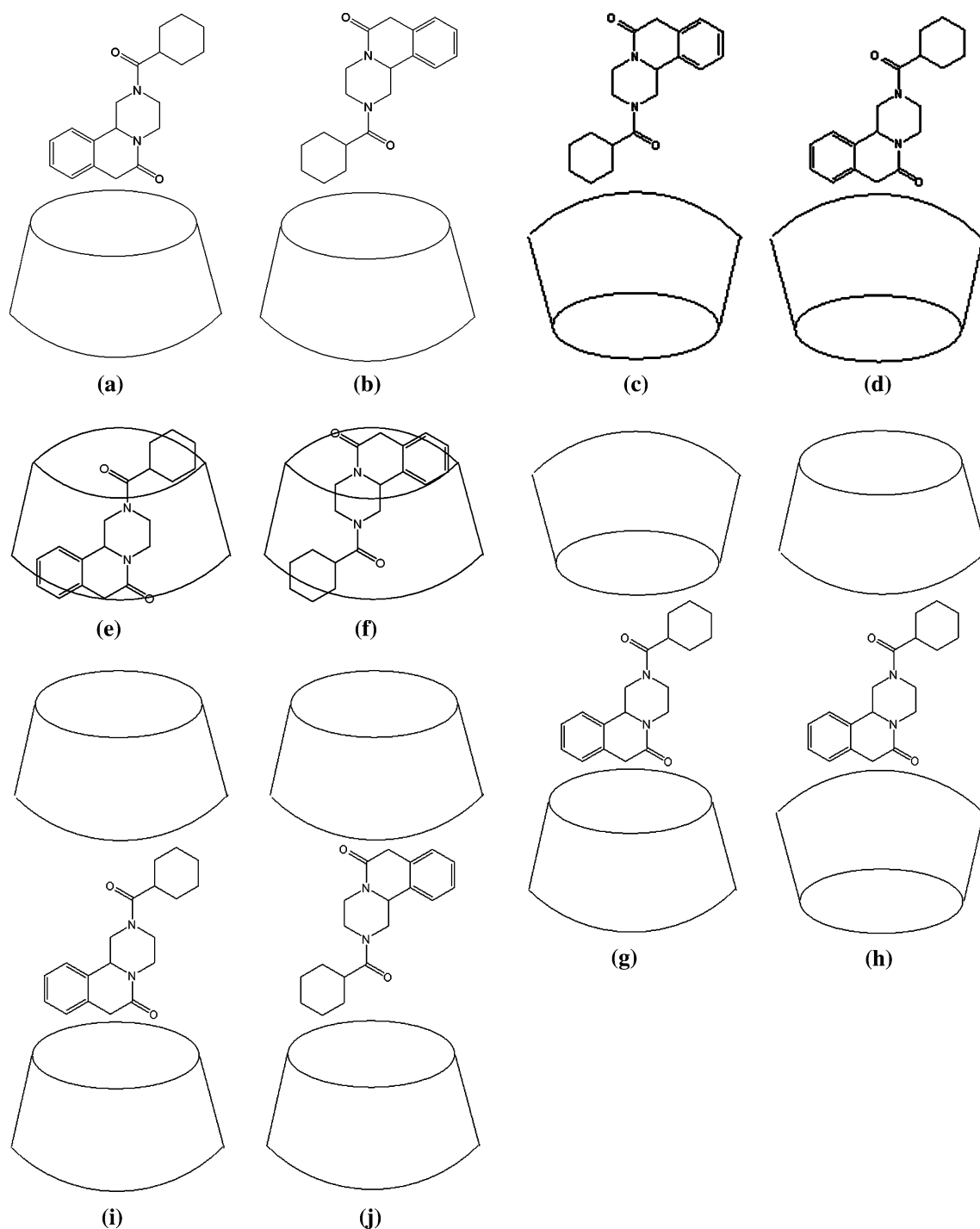
Semi-empirical methods have been suggested to be suitable and very convenient for the modeling of large molecular systems, requiring negligible computational facilities compared to ab initio methods [14, 17–19]. Here, they were employed to predict the best fit of PZQ in the MeCD cavity. The theoretical calculations were performed using the Spartan software package [20]. The molecular geometries of MeCD, PZQ and complexes were fully optimized using the MMFF and PM3 semi-empirical methods, using Solvation Model 5.4 (SM5.4) [21].

The  $\beta$ -cyclodextrin structure was obtained from the Protein Data Bank (PDB code: 1DMB), and a methoxy group replaced each hydroxyl group bonded to C-2 and C-6, in order to give the 2,6-di-*O*-methyl- $\beta$ -cyclodextrin. The PZQ structure was built and optimized at molecular mechanics and semi-empirical levels using the Spartan program, and inserted inside the MeCD cavity manually. The inclusion compounds PZQ:MeCD (at 1:1 or 1:2 molar ratios) were fully optimized using the orientations shown in Fig. 2.

The binding affinities ( $\Delta H$ , kcal mol<sup>-1</sup>) were obtained using the equations below:

$$\text{PZQ:MeCD,} \\ \Delta H = \Delta H_{\text{f(PZQ:MeCD)}} - (\Delta H_{\text{f(PZQ)}} + \Delta H_{\text{f(MeCD)}}) \quad (1)$$

$$\text{PZQ:2MeCD,} \\ \Delta H = \Delta H_{\text{f(PZQ:2MeCD)}} - (\Delta H_{\text{f(PZQ:MeCD)}} + \Delta H_{\text{f(MeCD)}}) \quad (2)$$



**Fig. 2** Schematic representation of the starting orientations of PZQ:MeCD used in the molecular modeling at 1:1 (a, b, c, d, e, f) and 1:2 (g, h, i, j) molar ratios

#### Preparation of the PZQ:MeCD inclusion compound

Solid inclusion compounds were obtained by mixing appropriate amounts of PZQ (Merck) and MeCD (gift from Roquette, Lestrem, France, with an average degree of substitution of  $DS = 0.5$ ) in a 1:1 molar ratio, using the

methodology described by de Jesus et al. [15]. Briefly, PZQ was solubilized in acetone, and MeCD in ultra-pure water (Millipore), and after stirring, they were submitted to vacuum to remove all the solvent. The product obtained was re-suspended in water, freeze-dried, and stored at  $-20\text{ }^{\circ}\text{C}$  before use.

## Characterization of the solid PZQ:MeCD inclusion compound

Solid inclusion compounds were investigated using scanning electron microscopy (SEM), infrared spectroscopy (IR) and differential scanning calorimetry (DSC), in order to obtain information concerning inclusion complex formation. Samples of plain PZQ, plain MeCD, and the inclusion compound PZQ:MeCD (1:1 molar ratio) were analyzed in these experiments. Physical mixtures were also prepared and used as controls.

The morphology of the inclusion compound obtained was determined using a scanning electron microscope (LEO EZO40). Samples were prepared on aluminum stubs, using double-sided sticky tabs, and vacuum-coated with gold for 180 s, to render them electrically conductive.

Infrared spectroscopy was performed with an Excalibur Series FTS 3000 spectrometer, using a wave number range from 4000 to 400  $\text{cm}^{-1}$ , 16 overlaps and resolution of 4  $\text{cm}^{-1}$ . Samples were homogenized with KBr and mounted into the holder using a compression gauge.

A Shimadzu DSC-60 calorimeter was used for the differential scanning calorimetric analyses. Approximately 4 mg portions of the samples were heated at a rate of 10  $^{\circ}\text{C min}^{-1}$ , from 30 to 200  $^{\circ}\text{C}$ , under a nitrogen atmosphere. Indium was employed to calibrate the instrument.

## Analysis of the PZQ:MeCD inclusion compound in solution

UV spectrophotometry was used in investigations of a solution containing the PZQ:MeCD inclusion compound, at a 1:1 molar ratio. NMR spectroscopy provided direct information of molecular recognition in this inclusion compound.

### The solvent polarity effect

Comparison of the behavior of PZQ under different conditions of environmental dielectric constant [22] was performed by dissolving appropriate amounts of the compound in water, ethanol or a solution of MeCD, to give identical concentrations. The spectrum was then recorded from 200 to 300 nm, for each solution.

### Inclusion kinetics

The kinetics of the inclusion was explored in order to estimate the equilibrium time for the complexation process. Appropriate amounts of PZQ and MeCD (1:1 molar ratio) were mixed in water, at 30  $^{\circ}\text{C}$  with stirring. Aliquots were withdrawn from the solution and analyzed in triplicate (at 270 nm), until stabilization of the absorbance

signal. Normalization was performed for each value (normalized absorbance = absorbance of PZQ at time  $t$ /absorbance of PZQ at time zero). The kinetic plot was analyzed using a zero-order rate, a first-order rate and a second-order rate, to obtain the kinetic constant of complexation [23].

### Phase solubility diagrams

Phase solubility diagrams were constructed according to the method described by Higuchi and Connors [24]. An excess amount of PZQ (8 mM) was added to Erlenmeyer flasks containing increasing concentrations of MeCD (from 0 to 35 mM), and the flasks were then sealed and stirred (at 30  $^{\circ}\text{C}$ ) until equilibrium was achieved. The suspensions were centrifuged for 30 min at 280 g, and then filtered through 0.22  $\mu\text{m}$  membrane filters (Millipore). The concentration of PZQ was determined spectrophotometrically, at 270 nm. The association constant ( $K_a$ ) was calculated from the linear plot obtained in the diagram, using the Eq. 3 proposed by Higuchi and Connors [24]. The experiment was carried out in triplicate.

$$K_a = \frac{\text{slope}}{S_0(1 - \text{slope})} \quad (3)$$

where  $S_0$  = PZQ solubility in water, without MeCD.

### NMR spectroscopy

$^1\text{H}$  experiments were acquired with a MERCURY-300 Varian spectrometer operating at 300.068 MHz for  $^1\text{H}$  (64K data points, 45 $^{\circ}$  excitation pulse duration of 2.2  $\mu\text{s}$ , spectral width of 6 kHz, acquisition time of 3.6 s and relaxation delay of 2 s) in a 5 mm probe with inverse detection mode. Samples of plain PZQ, plain MeCD and PZQ:MeCD inclusion compound (1:1 molar ratio) were recorded at 25  $^{\circ}\text{C}$  in  $\text{D}_2\text{O}$ . The residual water signal was used as the internal reference at 4.7 ppm.

### Cell culture and cytotoxicity assays

3T3 Chinese hamster lung fibroblasts were routinely grown in DMEM containing antibiotics (100  $\text{U mL}^{-1}$  penicillin G; 100  $\mu\text{g mL}^{-1}$  streptomycin) and supplemented with 10% foetal calf serum, in a 5%  $\text{CO}_2$  humidified atmosphere, at 37  $^{\circ}\text{C}$  [25].

For the cell viability assays, 96-well tissue culture plates were inoculated with  $3 \times 10^4$  cells  $\text{mL}^{-1}$ , and incubated at 37  $^{\circ}\text{C}$  for 48 h. The medium was removed and replaced with treatment medium containing different doses of the test material. The cells were then incubated for 24 h [26]. Different concentrations of PZQ, MeCD or 1:1 PZQ:MeCD inclusion compound were tested (0.2–0.9 mM). All of the

experiments were run using eight replicates. Cell viability was assessed by the MTT reduction assay.

The medium containing treatments was removed, and 0.1 mL of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) solution (0.5 mg mL<sup>-1</sup> of serum-free culture medium) was added to each well. After incubation for 4 h at 37 °C, the medium was removed and the formazan crystals solubilized in 0.1 mL of ethanol. The solutions were homogenised for 5 min on a plate shaker, and the absorbance then measured at 570 nm [27].

## Results and discussion

### Computational analysis

It was previously demonstrated that molecular mechanics showed good agreement with experimental data in determination of the PZQ:β-CD inclusion compound geometry [14]. This theoretical tool was used here to establish the PZQ (guest)–MeCD (host) interaction geometry.

According to the docking analysis performed, all the possibilities for the PZQ entrance into the methyl-β-cyclodextrin cavity were tested, but not using additional tools such as the Monte Carlo method. However, the theoretical results are consistent with NMR data and other studies [14, 28].

Table 1 shows the values of the formation enthalpies ( $\Delta H_f$ ) and binding affinity energies for different possible interactions, calculated according to Eq. 1, for the PM3 and MMFF methods. The negative binding affinity values for the PM3 calculations indicated that five orientations were stable in water. The MMFF results revealed that all the possibilities were stable in water.

**Table 1** Formation enthalpies and binding affinities (kcal mol<sup>-1</sup>) obtained using semi-empirical PM3 and MMFF calculations for PZQ, MeCD and inclusion compounds

Compound	PM3		MMFF	
	$\Delta H_f$	Binding affinity	$\Delta H_f$	Binding affinity
PZQ	-81.7	-	64.6	-
MeCD	-1404.6	-	-650.6	-
<b>a</b>	-1516.1	-29.8	-1344.8	-758.8
<b>b</b>	-1496.0	-9.7	-1367.1	-781.1
<b>c</b>	-1511.0	-24.7	-1253.8	-667.8
<b>d</b>	-1474.4	11.9	-1235.5	-649.5
<b>e</b>	-1487.8	-1.5	-1317.3	-731.3
<b>f</b>	-1511.3	-25.0	-1322.9	-736.9

Solvation calculations were performed using SM5.4/A (Solvation Model 5.4)

For the 1:1 complexes, the most stable were the **a** orientation ( $-29.8$  kcal mol<sup>-1</sup>) for PM3, and the **b** orientation ( $-781.1$  kcal mol<sup>-1</sup>) for MMFF (Fig. 2). Both of these involve the insertion of PZQ via the non-methylated border of MeCD (Fig. 3). PZQ interaction with the methylated border of MeCD is not as stable as with the non-methylated border, as shown by **c** in the MMFF calculation, and **d** in the MMFF and PM3 calculations. These results demonstrate that the methyl groups could decrease the interaction between PZQ and MeCD.

The reason for the differences between PM3 and MMFF results concern the appropriate differences between semi empirical and molecular mechanics calculations. In the case of semi empirical the atoms in the molecules are described from the results of Schrodinger equations. For molecular mechanics the atoms are like little spheres and only Newton aspects are considered.

1:2 (guest:host) stoichiometry calculations were performed, with the values of the formation enthalpies and binding affinity energies for the different possible interactions determined according to Eq. 2. The 1:2 orientations were possible to calculate under vacuum (data not shown), but not in solution, indicative of an unstable complex in solution, in agreement with previous results that have found the best PZQ:CDs interactions at a 1:1 molar ratio [13–16].

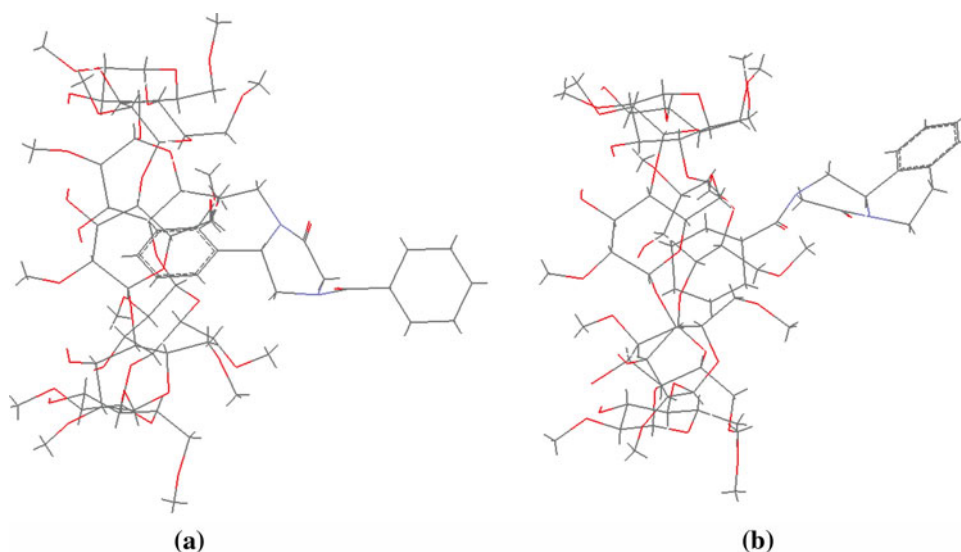
### Characterization of the solid PZQ:MeCD inclusion compound

A complexation study in the solid state for the PZQ:MeCD inclusion compound was performed using SEM, IR and DSC.

Figure 4a and b show the crystalline features of the plain PZQ and MeCD, in SEM images obtained at the same magnification ( $\times 1500$ ). The MeCD crystals are larger, with a smooth surface. The PZQ crystals are small and elongated, with sharp edges, as described by Liu et al. [29]. A change in the morphological characteristics was observed after inclusion, with formation of an amorphous powder (Fig. 4c). Physical mixtures, scanned as controls (data not shown), did not lose the crystalline characteristics of the plain samples. It was therefore clear that the inclusion compounds had been formed.

Formation of an inclusion compound can be investigated by IR spectroscopy, since the absorption bands of the guest molecule change in frequency and intensity after interaction with the CD cavity [30, 31]. Figure 5 shows IR absorbance spectra for the samples tested. The plain PZQ spectra are quite different from the spectra for plain MeCD and PZQ:MeCD, with sharp peaks characteristic of a smaller organic molecule. PZQ shows C–H and C–H<sub>2</sub> stretching vibration peaks at 3028 and 2954 cm<sup>-1</sup>, and a

**Fig. 3** PZQ:MeCD in 1:1 molar ratio by **a** PM3 and **b** MMFF calculation



carbonyl (amide group of praziquantel) stretching vibration at  $1736\text{ cm}^{-1}$ . The peaks in the region  $3325\text{--}3738\text{ cm}^{-1}$ , and in the region below  $1535\text{ cm}^{-1}$  (C=C stretching), confirm the racemic form of the drug, and are in good agreement with the literature [32]. It is also possible to identify a C–N stretching at  $1205\text{--}1346\text{ cm}^{-1}$ , as well as a =C–H bend peak at  $758\text{ cm}^{-1}$ , characteristic of the PZQ aromatic ring.

The spectrum for the physical mixture (Fig. 5a) does not show any noticeable changes compared to the spectra for the plain molecules. Figure 5b shows PZQ and MeCD patterns, separately, suggesting a lack of interaction between them in the physical mixture.

Slight changes can be observed in the PZQ:MeCD inclusion compound spectrum. There is a decrease in the plain MeCD band at  $3000\text{--}3500\text{ cm}^{-1}$ , compared with the complex, probably due to the breaking of hydrogen bonds of the hydration water molecules inside the CD cavity, after insertion of the guest (PZQ). The PZQ spectrum also shows minor changes, with decrease of C–N stretching at  $1205\text{--}1346\text{ cm}^{-1}$  after inclusion, and a visible effect on the aromatic ring C–H at  $758\text{ cm}^{-1}$ . This topology is in agreement with the molecular mechanics data, as well as with the PZQ: $\beta$ -CD inclusion compound previously described by de Jesus et al. [14].

Thermograms obtained by DSC show changes in the transition temperatures after inclusion of the PZQ molecule into the MeCD cavity (Fig. 6). The plain MeCD thermogram shows a broad transition, due to the loss of cavity water molecules. PZQ presents an endothermic peak with transition temperature near  $140\text{ }^\circ\text{C}$ , corresponding to its melting point [15]. After the inclusion procedure, the PZQ molecule loses its transition, demonstrating formation of the inclusion compound, as observed for other molecules

[33]. The physical mixture showed peaks remaining, demonstrating that there was no full inclusion.

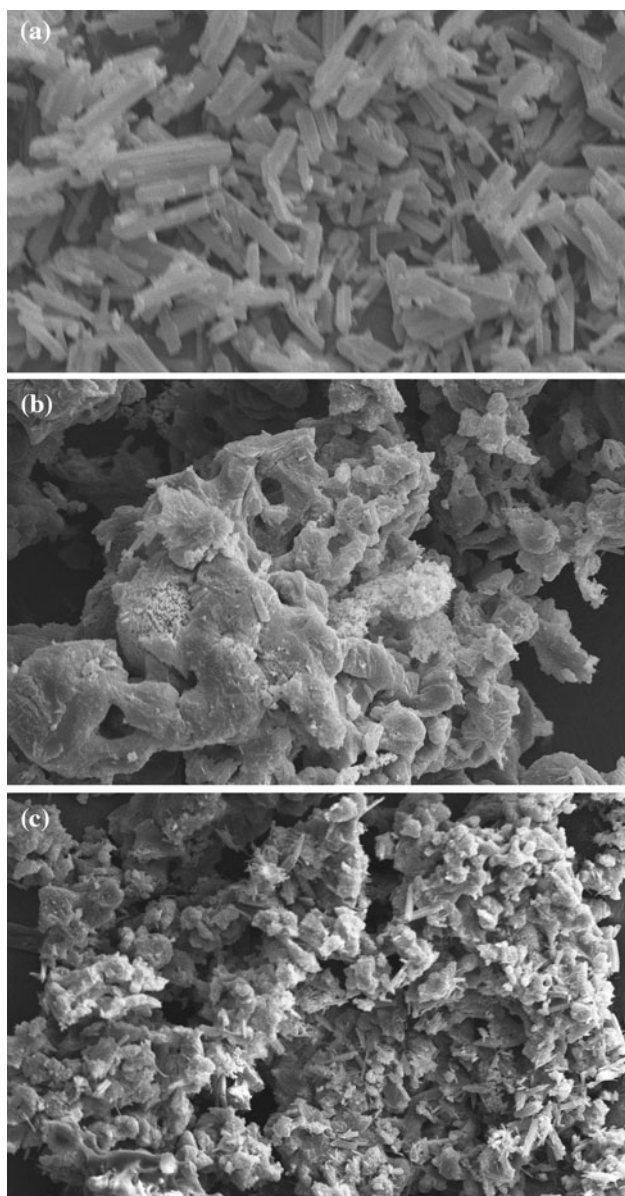
The DSC measurements therefore supported the SEM and infrared spectroscopy data, strengthening the evidence for PZQ:MeCD inclusion compound formation in the solid state, at the molar ratio tested.

#### Analysis of PZQ:MeCD in solution

Many molecules have their physico-chemical profiles altered after molecular inclusion, which are reflected in changes in the spectra obtained by UV/Vis spectrophotometry [22]. Such changes can be observed using different polarity solvents, where the drug could move to a less polar environment, such as the CD cavity [34]. The alterations may be caused by electronic clouds disturbed by the direct interaction with CD, exclusion of water molecules from the CD cavity, or both mechanisms [35, 36]. Figure 7 shows the change in the intensity of the signal due to the different dielectric constants of the solvents tested. When PZQ is inserted into the MeCD cavity, the environment has a polarity similar to that of an ethanolic solution [22, 37, 38].

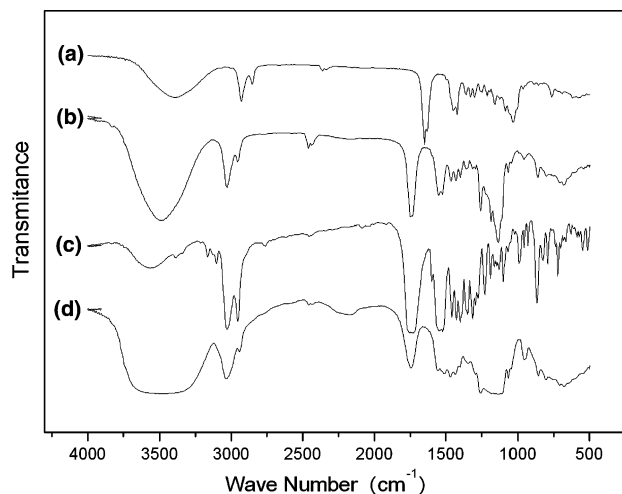
Direct evidence of inclusion compound formation was obtained from changes in the PZQ:MeCD UV spectrum. PZQ presents a bathochromic shift in the spectrum in the presence of a solvent such as ethanol, which is less polar compared to water. When an aqueous solution of MeCD is used, the bathochromic shift is also observed, with a hypochromic effect, as described for carotene: $\beta$ -CD inclusion compounds [22].

Spectroscopic changes of PZQ in the presence of MeCD (1:1 molar ratio) were recorded, at regular intervals, until equilibrium was reached (26 h). Figure 8 presents the kinetic plot obtained during the inclusion compound formation

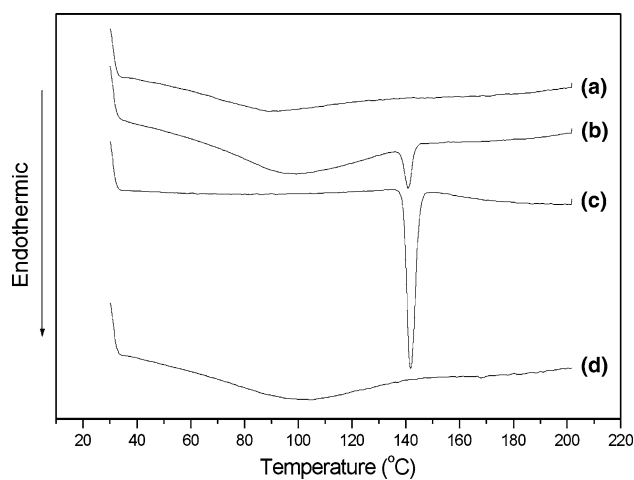


**Fig. 4** Scanning electron micrography of **a** PZQ, **b** MeCD and **c** PZQ:MeCD at 1:1 molar ratio and at the same magnification ( $\times 1500$ )

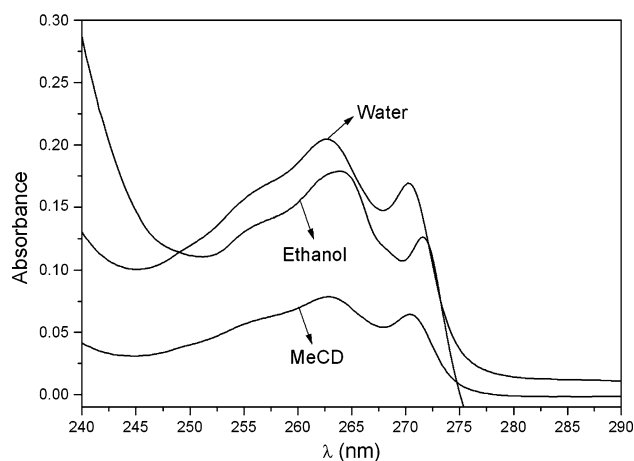
experiment. Values were normalized to facilitate comparison (normalized absorbance = absorbance of PZQ at time  $t$ / absorbance of PZQ at time zero). During this assay, no change was observed in the maximum wavelength, but only in its intensity. This increase in absorbance value was observed up to 10 h, when stabilization occurred. Analysis of the kinetic profile was performed with Origin<sup>®</sup> version 7 software. The best fit for the results of absorbance as a function of time ( $r = 0.95$ ) was indicative of zero order complexation kinetics. The kinetic constant value ( $k$ ) was determined to be  $0.147 \text{ h}^{-1}$ . Similar results have been observed using hydroxypropyl-beta-cyclodextrin (HP- $\beta$ -CD)



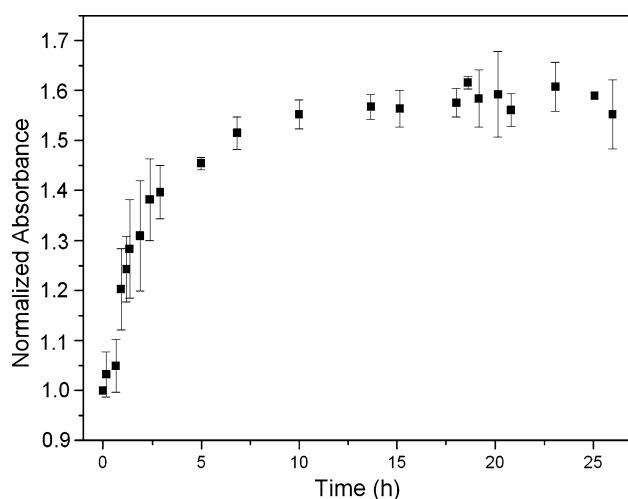
**Fig. 5** Infrared spectroscopy for **(a)** Physical mixture; **(b)** PZQ:MeCD inclusion compound; **(c)** plain PZQ; **(d)** plain MeCD



**Fig. 6** Differential scanning calorimetry for **(a)** PZQ:MeCD inclusion compound; **(b)** physical mixture; **(c)** plain PZQ; **(d)** plain MeCD

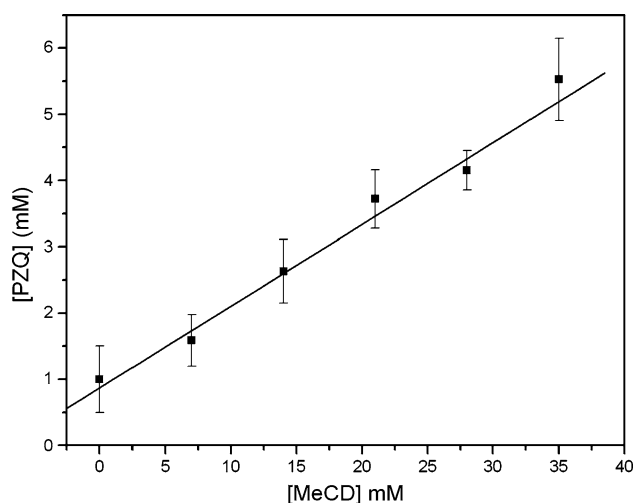


**Fig. 7** Polarity assessment of PZQ inserted into different environments: water, ethanol and aqueous solution of MeCD. All samples were prepared at the same concentration



**Fig. 8** Kinetic plots of PZQ:MeCD complexation at 1:1 molar ratio and PZQ, where the equilibrium time for inclusion complex formation was 8 h, and the kinetic constant value was  $0.183 \text{ h}^{-1}$  [16].

After the equilibrium time had been established, the phase solubility isotherm was used to evaluate the solubilization of PZQ with MeCD (Fig. 9). The solubility of PZQ increased with increasing MeCD concentration, from 1.0 mM (with no MeCD) to 5.53 mM (a 5.5-fold gain). A type  $A_L$  isotherm was observed, with a linear increase in the solubility, characteristic of a 1:1 molar ratio. The linear fit was used to obtain an association constant ( $K_a$ ) of  $140.8 \text{ M}^{-1}$ . For the interaction of PZQ with  $\beta$ -CD, de Jesus et al. [14] and Becket et al. [13] obtained  $K_a$  values of 440.83 and  $396.91 \text{ M}^{-1}$ , respectively. A decrease in the value could be related to the modified CD used. The presence of methoxy groups could hinder access of PZQ to



**Fig. 9** Phase solubility isotherms for praziquantel with increasing concentrations of MeCD

the MeCD cavity, resulting in a less favorable interaction than observed with  $\beta$ -CD. Nonetheless, the value is large enough to ensure that the molecule interacts with the cavity, prolonging its release. For HP- $\beta$ -CD, the value obtained was  $47.29 \text{ M}^{-1}$  [16], demonstrating that with hydroxypropyl groups, the steric hindrance is more pronounced than with methyl groups. It may also be relevant that the MeCD used (CRYSMEB) had a lower degree of substitution (DS = 0.5; manufacturer's data) compared to HP- $\beta$ -CD (DS = 0.6).

For  $\beta$ -CD and derivatives, the  $K_a$  values were in the following order: HP- $\beta$ -CD <  $\beta$ -CD < DM- $\beta$ -CD, probably due to significant enhancement of the complexation ability caused by methylation, which makes the cyclodextrin environment more hydrophobic and assists the CD-guest interaction [39].

In attempt to investigate the interaction of PZQ with MeCD and evidence their complexation  $^1\text{H}$  NMR spectroscopy was employed. NMR is a tool widely used to evaluate the inclusion of host molecules in the CD cavity [35, 40]. Assignments of hydrogen NMR peaks are in good agreement with the literature, for MeCD [41] and PZQ [14]. Differences in the chemical shifts of some PZQ hydrogens after inclusion were recorded, denoting a change in the environment, as demonstrated here with other techniques.

For MeCD there is only a slight change in the chemical shifts, after the inclusion of PZQ (data not shown). These findings seem to be consistent with other research which found that hydrogens do not experience a significant shift because of the small molecule included [40].

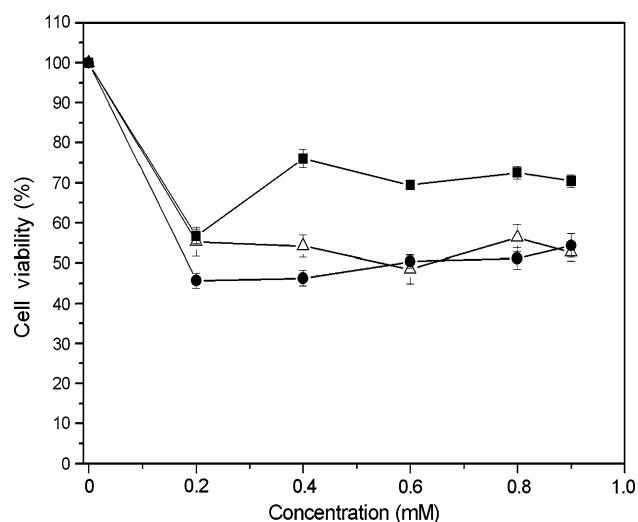
The most affected hydrogens of PZQ after inclusion into the MeCD were 6a, 3a and 14 (Table 2 and Fig. 1a). It demonstrates that the isoquinoline ring of PZQ is inserted in the cavity of MeCD, which confirm the molecular recognition. This result also corroborate the findings of theoretical calculations and spectroscopic experiments indicating an 1:1 stoichiometry between PZQ:MeCD.

**Table 2** Effect of MeCD on  $^1\text{H}$  NMR chemical shifts of PZQ in  $\text{D}_2\text{O}$  at 25 °C

Hydrogen position	PZQ chemical shift (ppm)	PZQ:Me-CD chemical shift (ppm)	$\Delta\delta$ (ppm)
H-6a	3.08	2.96	0.12
H-1a	3.41	nd	nd
H-3a	3.85	3.98	-0.13
H-6b	4.17	4.19	-0.02
H-14	4.98	4.86	0.12

nd not determined because of peak sobreposition





**Fig. 10** Cell viability assessed by MTT endpoint in different concentrations of PZQ:MeCD 1:1 (filled square), PZQ (open triangle) and MeCD (filled circle)

### Cytotoxicity assays

Cell viability was analyzed using the MTT assay for plain PZQ, plain MeCD and the PZQ:MeCD (1:1) inclusion compound (Fig. 10). At the concentrations tested (0.2–0.9 mM), none of the solutions appeared to increase the toxicity to 3T3 cells. Inclusion into the MeCD cavity did not result in profiles different to those observed for the plain molecules. Hence, the PZQ:MeCD inclusion compound did not exhibit cytotoxicity to these cells, corroborating previous results reported by de Jesus et al. [14] and Chaves et al. [16].

### Conclusions

A physico-chemical characterization of praziquantel inserted into a methoxylated  $\beta$ -cyclodextrin was performed. Molecular modeling results and Monodimensional  $^1\text{H}$  NMR suggested that PZQ was inserted into MeCD with a 1:1 stoichiometry, via the non-methylated border. IR results corroborated these findings, and showed that the PZQ aromatic ring was affected by the presence of MeCD. Morphological and calorimetric analyses demonstrated successful formation of the inclusion compound, and the insertion of PZQ into a less polar environment was assessed in spectroscopy experiments. A fivefold increase in solubility, observed after inclusion, could render PZQ more bioavailable.  $^1\text{H}$  NMR experiments also provided the direct information of molecular recognition between PZQ and MeCD. Preliminary in vitro assays confirmed that the material did not present toxicity to fibroblast cells, at the concentrations tested. Alternative administration routes in

humans could therefore be employed using this system, which might be useful in cases of more severe illness or in different posological schemes. Future work should include pharmacological investigations using the PZQ:MeCD formulation.

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