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Research Article

Solving the Delivery Problems of Triclabendazole Using Cyclodextrins

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Abstract. Triclabendazole is the first-line drug of choice to treat and control fasciolasis, a neglected parasitic human disease. It is a class II/IV compound according to the Biopharmaceutics Classification System. Thus, the aim of this study was to improve aqueous solubility and dissolution rate of triclabendazole complexed with 2-hydroxylpropyl-β-cyclodextrin (HP-β-CD) and methyl-β-cyclodextrin (Me-β-CD) at 1:1 and 1:2 M ratio. The impact of storage on the solubility, dissolution profile, and solid-state properties of such complexes was also investigated. Drug-carrier interactions were characterized by infrared spectroscopy, differential scanning calorimetry, X-ray diffractometry, and scanning electron microscopy. The solubility of triclabendazole improved up to 256- and 341-fold using HP-β-CD and Me-β-CD, respectively. In particular, the drug complexed with Me-β-CD showed a positive deviation from linearity, suggesting that its solubility increases with an increasing concentration of Me-β-CD concentration in a nonlinear manner. The drug dissolution was found to be improved through complex formation with HP-β-CD and Me-β-CD. In particular, the 1:2 M ratio complexes exhibited higher dissolution than the corresponding 1:1 M ratio complexes. The physicochemical characterization of the systems showed strong evidence of amorphous phases and/or of the formation of an inclusion complex. Stored at 25 °C, 60% RH for 24 months, drug complexed with β-cyclodextrins (CDs) at 1:2 M ratio remained amorphous. Based on these findings, it is postulated that the formation of triclabendazole-CD inclusion complexes produced significant enhancement in both the dissolution and solid-state properties of the drug, which may lead to the development of triclabendazole novel formulations with improved biopharmaceutical characteristics.

KEY WORDS: triclabendazole; cylodextrin; amorphous nature; dissolution profiles; storage.

INTRODUCTION

Fascioliasis is a neglected tropical disease caused by the trematode species *Fasciola hepatica* and *Fasciola gigantica*. As reported, more than 2.5 million people are infected in different regions of Africa, America, Asia, and Europe while more than 80 million are at risk (1,2). The symptoms of the disease may include extensive hepatic tissue damage, severe anemia, and intense inflammatory reactions in response to the migration of immature parasites and the feeding of mature flukes (1,3). On the other hand, *Fasciola* spp. is also a serious concern in veterinary medicine, particularly in sheep and cattle, producing significant economic losses *per annum* in animal production (4). Animals get the infection by ingesting infected plants while human infections are produced by consuming infected raw aquatic vegetables and/or water (5). Although the number of infected population is growing in several endemic regions due to a combination of economic, environmental, and sociocultural reasons, triclabendazole (TCBZ) (Fig. 1), included in the WHO Essential Drug List, is still the only chemotherapeutic agent widely used in both human and veterinary medicine (6,7).

However, resistance to TCBZ has been documented in many countries and, as a consequence, the discovery of novel chemotherapeutic agents with improved anthelmintic activity is necessary (8,9). In this regard, novel treatments against *Fasciola hepatica* and *Gigantica* based on artemisinin derivatives (10,11), ivermectin (12), and oxfendazole (13) were recently reported. Even though some promising results were obtained with these agents, additional studies are needed to determine if such chemotherapeutic alternatives could achieve parasitological cure in humans. TCBZ is poorly water

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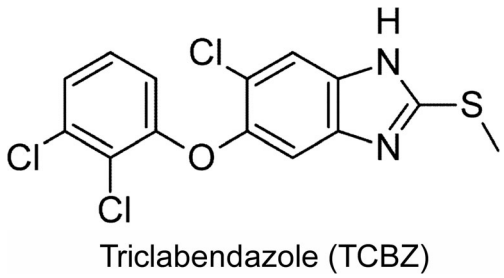


Fig. 1. Triclabendazole (TCBZ) structure

soluble (0.24 μg mL^{-1}) and highly lipophilic (log *P* of 5.44) thus being classified as BCS class II/IV (14). Similarly to other poorly water-soluble anthelmintic benzimidazoles (15–17), its very low aqueous solubility is a major concern regarding the developing of novel formulations with appropriate biopharmaceutical properties. To overcome such a drawback, it was recently reported the synthesis of a TCBZ water-soluble phosphate salt prodrug. It was found that such new derivative had a remarkable increase of solubility in water. This prodrug was effectively converted into TCBZ in basic conditions and the corresponding *in vivo* studies indicated that, after intramuscular administration, the TCBZ prodrug exhibited a similar fasciolicidal efficiency compared with the commercial available TCBZ suspension (18). However, no data was shown related to the oral administration of such prodrug. On the other hand, a complex between TCBZ and β-cyclodextrin (CD) was applied to the treatment of a parasitic infection caused by *Ichthyophthirius multifiliis* in rainbow trout (19). Even though the inclusion complex exhibited a limited aqueous solubility, its solubility was 16 times higher than that of the noncomplexed drug. *In vivo* studies demonstrated that the infection was drastically diminished in animals treated with complexed TCBZ in comparison with those treated with raw TCBZ, suggesting that β-CD is a convenient carrier to enhance the solubility and further biological efficacy of TCBZ. Cyclodextrins, a group of cyclic oligosaccharides, consist of (α-1,4)-linked α-D-glucopyranose units and contain a lipophilic central cavity and a hydrophilic outer surface (20,21). Due to their particular lipophilic/hydrophilic structure, CDs have the capability to form inclusion complexes with hydrophobic drugs leading, usually, to an increase of aqueous solubility, dissolution rate, stability, and further bioavailability of such lipophilic molecules (22,23). Even though CDs have been widely applied to increase the solubility and biopharmaceutical performance of several benzimidazoles (24–26), there is a lack of information related to the behavior of TCBZ after complexation with CD derivatives, specifically in terms of solubility, dissolution rate, and stability. Therefore, this paper reports the impact of stoichiometric and nonstoichiometric TCBZ-CD complexes on the physicochemical properties, solubility, and dissolution rate of TCBZ. TCBZ:2-hydroxylpropyl-β-cyclodextrin (HP-β-CD) and TCBZ:methyl-β-cyclodextrin (Me-β-CD) complexes (1:1 and 1:2 M ratio) were characterized by differential scanning calorimetry (DSC), X-ray diffractometry (XRD), infrared spectroscopy (IR), and scanning electron microscopy (SEM). In addition, TCBZ:HP-β-CD and TCBZ:Me-β-CD samples were investigated after storage for 24 months in terms of solubility, dissolution, rate, and crystalline state.

MATERIALS AND METHODS

Materials

TCBZ (lot PLP 40000140, 99.80% purity) was purchased from CHEMO Argentina (Buenos Aires, Argentina). β-Cyclodextrin (β-CD), hydroxypropyl-β-cyclodextrin (estimated molecular weight (mol wt), ~1380; average degree of substitution, 0.6; HP-β-CD), and methyl-β-cyclodextrin (estimated mol wt, 1310; average degree of substitution, 1.8; Me-β-CD) were purchased from Sigma-Aldrich (Saint Louis, MO, USA). All the solvents and reagents were of analytical grade. Double-distilled water was used throughout the study.

Methods

Phase Solubility Studies

Phase solubility studies for TCBZ alone and complexed with CDs were performed using the procedure already described (27,28). Briefly, excess amount of TCBZ (100 mg) was added to each flask containing 10 mL of water or an aqueous solution of each CD (0–90 mM). The hermetically sealed flasks were shaken on a shaker (180 rpm) during 72 h in a water bath at 25 ± 0.5 °C. Then, the suspensions were filtered through cellulose nitrate membranes (0.45 μm pore size), and the concentration of TCBZ in solution was determined by UV spectrophotometry at 305 nm (Boeco S-26 spectrometer, Hamburg, Germany). Each experiment was carried out in triplicate. The stability constant (K_f) was calculated from the initial linear region of the phase solubility diagram, according to the Eq. (1)

$$K = \frac{\text{slope}}{S_0(1-\text{slope})} \tag{1}$$

where *S* is slope and S_0 is solubility of the TCBZ in the absence of CD.

Preparation of Physical Mixtures

Physical mixtures (PMs) of TCBZ:β-CD, TCBZ:HP-β-CD, and TCBZ:Me-β-CD were prepared at 1:1 and 1:2 M ratio by homogeneous blending in a mortar until a homogeneous mixture was obtained. All powder mixtures were prepared through the geometric dilution method. The powders were passed through a 250-μm mesh and stored in a desiccator until use.

Preparation of Inclusion Complexes

The inclusion complexes (IC) of TCBZ with β-CD, HP-β-CD, and Me-β-CD (1:1 and 1:2 drug:carrier molar ratio) were prepared by the solvent coevaporation procedure. Briefly, TCBZ (250 mg, 69 mmol) was dissolved in ethyl alcohol (10 mL) and CDs in water (10 mL). Then, CD solutions (69 or 138 mmol) were added to the TCBZ solution and stirred for 5 min. The solvents were removed by vacuum rotary evaporator (150 rpm) under reduced pressure

(15 mbar) at 40 °C. The solids were passed through a 250-μm mesh and stored in a desiccator until use.

Stability Studies

TCBZ-CDs PMs and IC samples were individually weighed and packed in amber-colored flask, stored at 25 °C 60% RH^{-1} in a pH 09 stability test chamber (Darwin Chambers Company, Saint Louis, MO, USA) for 24 months and evaluated in terms of solubility, dissolution rate, and solid-state properties.

Saturation Solubility Studies

The saturated solubility of TCBZ alone and TCBZ:CD samples (1:1 and 1:2 M ratio) were calculated by adding an excess amount (100 mg) of each sample to 100 mL solution of medium (distilled water, pH 6.3). The samples were shaken at 25 °C and 180 rpm in a Boeco orbital shaker (Hamburg, Germany) until an equilibrium was reached (72 h). Upon equilibrium, the samples were filtered through a 0.45-μm filter and measured by UV at 305 nm. All experiments were carried out in triplicate.

X-ray Diffraction.

Data collection was carried out in a Rigaku MiniFlex 600 diffractometer (Tokyo, Japan). X-ray diffraction (XRD) patterns were recorded using CuKα radiation ($\lambda =$ 1.540562 Å), a voltage of 40 kV, 15 A current, and steps of 0.025° on the interval $2\theta = 2°–5°$. Low peak broadening and background were assured by using parallel beam geometry by means of an X-ray lens and a graphite monocromator placed before the detector window. Data acquisition and evaluation were performed with the Stoe Visual-Xpow package, version 2.75 (Darmstadt, Germany).

Fourier-Transform Infrared Spectroscopy

Fourier transform infrared (FT-IR) spectra were obtained by an FT-IR Prestige-21. Shimadzu (Tokyo, Japan). The samples were prepared using the KBr disk method (2 mg sample in 100 mg KBr). Scanning range was 450 to 4000 cm^{-1} with a resolution of 1 cm^{-1}.

Differential Scanning Calorimetry

The thermal analysis of TCBZ and TCBZ complexed with HP-β-CD and Me-β-CD at 1.1 and 1:2 M ratio was performed using a Perkin-Elmer Pyris-1 DSC instrument (Waltham, MA, USA). Two samples of TCBZ and the corresponding inclusion complexes were used to evaluate the reproducibility of the thermal profile. Samples (4–7 mg) were placed in an aluminum sample pan and hermetically sealed. The samples were scanned at the heating rate of 10 °C min^{-1} over a temperature range of 30 to 250 °C. An empty pan was used as reference. An indium standard was used for calibration.

Scanning Electron Microscopy

The morphology of crystalline TCBZ and TCBZ-CD samples was analyzed by scanning electron microscopy (SEM) using an AMR 1000 Scanning Microscope (Amray, Bedford, MA). Samples were mounted on an aluminum sample support by means of a conductive and double-sided adhesive. Samples were previously sputter coated with a gold layer in order to make them conductive. Images were taken at 15 kV and × 500 magnification.

Dissolution Studies

Dissolution studies of TCBZ alone and from the TCBZ-CD samples were conducted according to US Pharmacopeia (USP) Apparatus 2 (Hanson Research, SR8 8-Flask Bath, Chatsworth, CA, USA). The dissolution medium was 900 mL of HCl 0.1 N maintained at 37 °C, and the stirring speed was set on 50 rpm. Each sample containing 90 mg of TCBZ was introduced into the flasks, and the time counter was set to zero. At different time intervals, 5 mL samples were withdrawn through a filter. The samples were assayed using an UV/Vis Boeco spectrophotometer at 305 nm. It was found that CDs did not interfere with the assay at this wavelength. The results presented are mean values of three determinations. Dissolution efficiency (DE), a concept proposed by Khan in 1975 (29) and defined as the area under a dissolution curve between specified time points, was calculated using the following equation:

$$DE\% = \frac{\int_o^t y \times dt}{y_0 \times 100} \times 100 \quad (2)$$

where y is the percentage of dissolved product at time t.

Statistical Analysis

Statistical significance of the differences between values was assessed by analysis of variance (ANOVA), and p values less than 0.05 were considered statistically significant (Statgraphics, Statistical Graphics System, Rockville, MA, USA).

RESULTS AND DISCUSSION

Phase Solubility Studies

The phase solubility diagrams are widely used to determine the inclusion ratio of inclusion complexes (30,31). In addition, it is very useful to analyze how drug solubility depends on the pH of the solution. As other related lipophilic benzimidazole derivatives, TCBZ may be ionizable at low pH values, and such ionization may greatly influence both further complexation and solubilization (32). However, it is also known that ionization of a molecule leads to the formation of less-stable complexes due to weaker interactions with the inner hydrophobic cavity of the CDs (33,34). Thus, in this study, the solubility of TCBZ with increasing concentrations of β-CD, HP-β-CD, and Me-β-CD, at different pH values was investigated (Fig. 2). In absence of CDs, it was found that

the solubility of TCBZ at pH 7 was $1.77 \cdot 10^{-3}$ mM while its solubility increased up to 82- and 163-fold using 60 mM of HP-β-CD and Me-β-CD, respectively. On the other hand, TCBZ exhibited a solubility of $4{,}7.10^{-3}$ mM at pH 3, which was increased more than 83-fold and 207-fold by adding HP-β-CD and Me-β-CD, respectively. Finally, at pH 1, TCBZ exhibited a solubility of 43.10^{-3} mM, which was dramatically enhanced up to 256-fold and 341-fold more using HP-β-CD and Me-β-CD, respectively. As observed in Fig. 2, TCBZ:Me-β-CD complex showed a positive deviation from linearity, at pH 3 and 7, indicating that the drug solubility increases with an increasing concentration of Me-β-CD concentration in a nonlinear manner in the range of 0–60 mM. This diagram could be classified as A_p that represents the formation of soluble complexes of second or higher order, suggesting the presence of both TCBZ:Me-β-CD complexes at 1:1 and 1:2 ratios (35). Particularly, at pH 7, the K_f value, obtained for the TCBZ:Me-β-CD inclusion complex was 1208.24 M^{-1}, indicating a relatively stable complex in comparison with the TCBZ:HP-β-CD (517.11 M^{-1}) and TCBZ:β-CD (77.77 M^{-1}). This finding could be related with the highly lipophilic character of the Me-β-CD cavity, which may provide a better environment for including lipophilic molecules such TCBZ (36). However, it is worth noting that at pH 1, it was observed that only one type of diagram was characterized by a straight line pattern (A_L-type system) indicating the formation of 1:1 type inclusion complex. Probably, at lower pH, the protonation of the imidazole ring of the TCBZ would increase the hydrophilic character of the drug affecting, as a consequence, the complexation with a second molecule of the carrier to form a 1:2 TCBZ:ME-β-CD inclusion complex. On the other hand, the phase solubility curve of the system TCBZ:HP-β-CD presents linear relationship between such components at pH 1, 3, and 7, indicating the formation of soluble 1:1 type inclusion complex (A_L-type system) (37). This finding is in agreement with the behavior of other related benzimidazoles complexed with CDs in acidic medium (38,39). As already described, the formation of inclusion complexes is due to the ability of the CDs to incorporate "guest" molecules into their truncated cone or "cup." Such a cone possesses an external hydrophilic surface decorated with hydroxyl groups and a hydrophobic inner cavity, formed by carbon and ether bonds. In the case of both HP-β-CD and Me-β-CD, the different substituent groups may produce steric hindrances at the entrance of the inner cavity avoiding or reducing, as a consequence, the interactions between the hydrophobic moiety of TCBZ and the CD cavity. In any case, the more lipophilic portion of TCBZ is incorporated into the hydrophobic cavity of the carrier giving the corresponding inclusion complex (40). Concerning the 1:2 drug:CD complexes, it should also be considered a complexation between one molecule of the guest TCBZ and two molecules of the host CD (31) (Scheme 1). Additionally, these findings are also consistent with those results obtained by di Cagno *et al.* (41) who demonstrated, by thermal analysis and NMR techniques, that Me-β-CD exhibits less steric hindrance than the HP-β-CD and, therefore, it may form stable complexes with ibuprofen. Concerning native β-CD, its complexes present also a limited aqueous solubility, even at pH 1 (42). As observed on this work, complexation with β-CD had no effect on the TCBZ solubility at the tested concentrations (0–40 mM). By increasing the amount of β-CD beyond 40 mM, a precipitate was observed, at all three pH assayed, suggesting that the aqueous solubility of β-CD (18.5 mg mL^{-1}) limits the formation of soluble inclusion complexes of poorly water-soluble compounds such as TCBZ, which was also demonstrated by Luzardo-Alvarez *et al.* (19). In agreement with these observations, TCBZ-Me-β-CD and TCBZ-HP-β-CD complexes were selected for further studies.

Saturated Solubility of the TCBZ Complexes

Once the inclusion complexes were characterized through the phase solubility diagram (Fig. 2), the solubility in water of both the PMs and IC of TCBZ with HP-β-CD and Me-β-CD (1:1 and 1:2 M ratio) as a function of the drug/CD ratio was analyzed (43). As observed in Table I, drug solubility was increased significantly even in PMs. The solubility of TCBZ:HP-β-CD and TCBZ:Me-β-CD (1:1 ratio) PMs were 0.01 and 0.008 mg mL^{-1}, respectively, while at 1:2 ratio, the values increased up to 0.02 and 0.01 mg mL^{-1}, respectively. Probably, in these samples, the improvement in drug wettability by means of CDs would reduce the powder agglomeration increasing the surface area and, as a consequence, the TCBZ aqueous solubility. Neither the type of carrier (HP-β-CD or Me-β-CD) nor the drug:carrier ratio (1:1 or 1:2) modified significantly the drug solubility. In contrast, a significant ($p < 0.05$) improvement in the aqueous solubility of TCBZ by complexation with such CD derivatives was observed. In particular, drug solubility from TCBZ:Me-β-CD (1:2 ratio) was found to be almost 500 times higher than the raw drug.

X-ray Diffraction

As reported, the modification of the crystalline patterns of a drug after complexation with cyclodextrins may affect their solubility, dissolution performance, stability, and

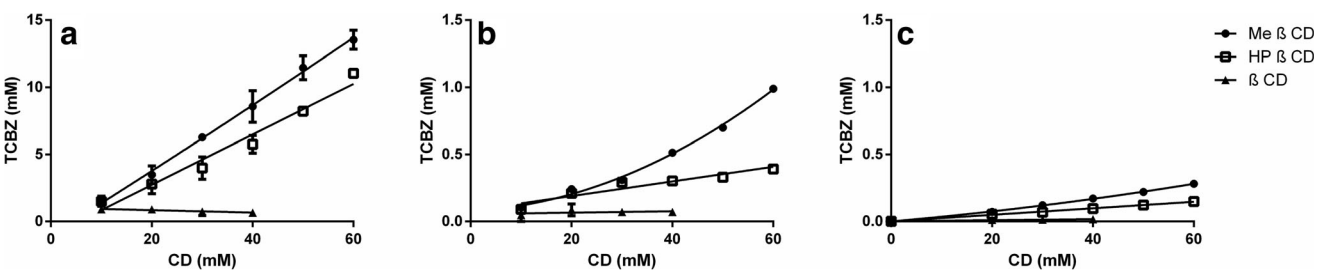
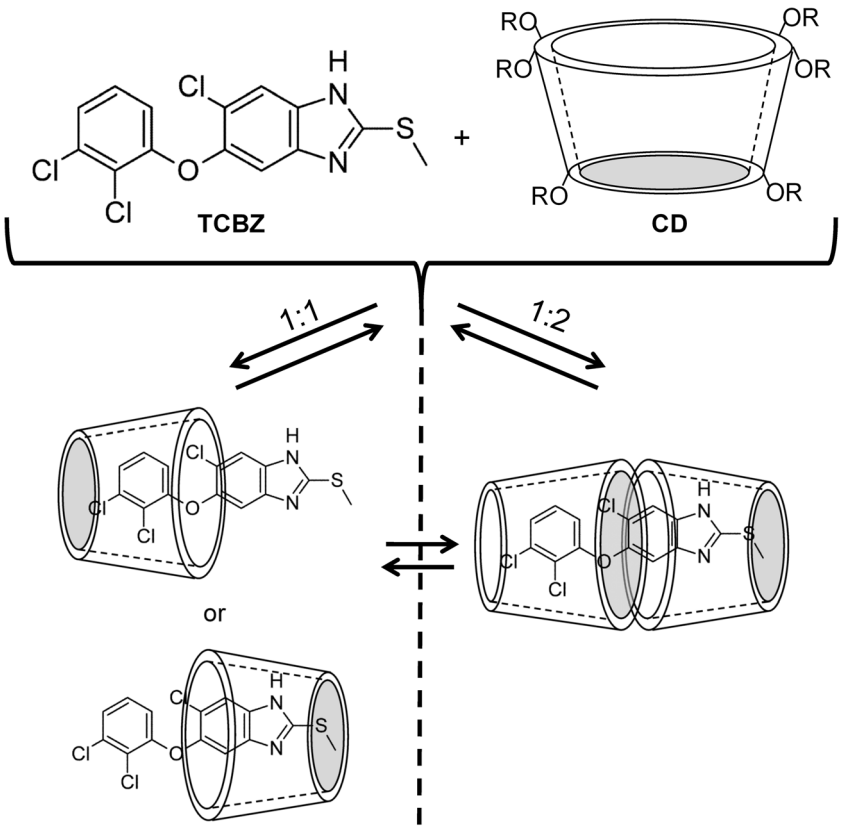


Fig. 2. Phase solubility diagram for TCBZ with increasing concentrations of β-CD, HP-β-CD, and Me-β-CD at pH 1 (**a**), pH 3 (**b**), and pH 7 (**c**). Values are mean ± SD ($n = 3$)



Name of CD	Structure of the substituent		Degree of molar sustitution
Me-β-CD	R= H and/or	CH_3	1,8
HP-β-CD		$CH_2CH(OH)CH_3$	0,6

Scheme 1. Graphical representation of the TCBZ complexation with CDs at 1:1 and 1:2 ratios

pharmacokinetic properties (22). In addition, a solid-state transition from crystalline to amorphous state of drugs may also be observed in these types of complexes after storage (44). Therefore, in this study, the analysis of both freshly and stored TCBZ/CD samples was evaluated by X-ray diffractometry. It is worth mentioning that no studies are available in the literature concerning it. The diffraction patterns of TCBZ and TCBZ/CD systems are shown in Fig. 3. In agreement with a previous report, it was possible to observe that TCBZ revealed its crystalline character showing a mixture of polymorphs (forms I and II) at 10.63, 12.89, 16.42, 17.94, 19.90, 23.71, 25.38, 25.86, 26.84, 27.82, and 31.36 (45). The analysis of both TCBZ:HP-β-CD and TCBZ:Me-β-CD PMs (1:1 and 1:2 M ratio) exhibited similar crystalline patterns, even though the intensity of the peaks was low compared with the nontreated drug. These patterns were found in both the fresh and aged samples, suggesting that the simple mixing between the components did not modified the crystalline state of the drug, even after 24 months at 25 °C 60% RH^{-1}. Such crystalline patterns may explain the poor solubility exhibited by these samples (Table I). On the other hand, the analysis of the TCBZ:Me-β-CD complex (1:1) showed an amorphous diffraction pattern which became slightly more crystalline after storage, while the TCBZ:Me-β-CD (1:2) complex exhibited an amorphous pattern not modified under storage. These data are in agreement with the results of the DSC analysis and confirmed the interactions between the drug and CDs. The analysis of both fresh and aged TCBZ:HP-β-CD complex (1:1) showed similar crystalline diffractograms, while opposite results were seen in case of fresh and aged TCBZ:HP-β-CD (1:2) complexes where an amorphous state of the drug was detected. Taking into account, it could be postulated that an excess of CD (1:2 M ratio) would be able to reduce or eliminate the recrystallization process. A similar finding was also reported in the complexation of spironolactone with HP-β-CD at 1:2 and 1:3 ratio. After storage at 60 °C, 75% RH, for 2 months, such carrier maintained an amorphous state of the drug (46).

Infrared Spectroscopy

The infrared spectrum of TCBZ, CD, and prepared samples are shown in Fig. 4. The characteristic bands of TCBZ appear at 914.26, 1045.42, 1085.92, 1155.36, 1257.59, 1338.60, 1375.25, 1419.61, 1456.26, 1575.84, 3419.79, and 3444.87 nm which are in good agreement with the data of

Table I. Aqueous solubility of the TCBZ:HP-β-CD and TCBZ:Me-β-CD samples

SYSTEM	Molar ratio	Sample	Solublity (mg mL^{-1})
HP-β-CD	1:1	PM	0.0105
		IC	0.0879
	1:2	PM	0.0204
		IC	0.1274
Me-β-CD	1:1	PM	0.0082
		IC	0.1186
	1:2	PM	0.0131
		IC	0.1694
TCBZ	–	RAW	0.0003

both the TCBZ forms I and II (45). The spectra of PMs and complexes showed differences and, also, an intensity reduction of mayor peaks of TCBZ. The major differences were found in the 700–1230 cm^{-1} region (Fig. 3), attributed to the skeleton vibrations of the C=C bonds in the aromatic ring and the aromatic C–N vibration. The intensity and shape of these bands changed dramatically for the inclusion compound as compared with those for nontreated TCBZ. As can be observed, peaks at 1381, 983, 874, 826, 767, 698, and 661 cm^{-1} disappeared in 1:2 TCBZ:HP-β-CD and TCBZ:Me-β-CD systems prepared by SD, while in complex 1:1 ratio, just peaks at 1381, 809, and 769 were absent using HP-β-CD and peaks at 983, 874, 828, 808, and 742 were missing when Me-β-CD was employed as carrier. The systems prepared by PM showed just a reduction in the intensity of the characteristic peaks of TCBZ probably due to the dilution of TCBZ in the carrier. On the other hand, the analysis of the drug spectra complexed with CDs confirm stronger interactions and the formation of inclusion complexes, as reported for other structurally related molecules (47).

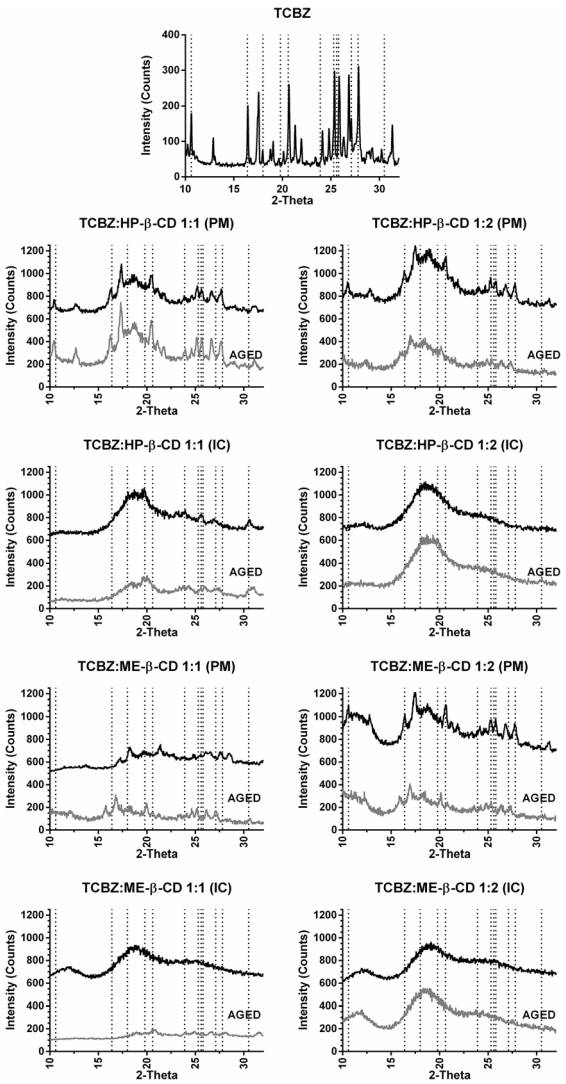


Fig. 3. X-ray diffraction patterns of TCBZ; TCBZ:CD physical mixtures (PM) and TCBZ:CD inclusion complexes (IC) at 1:1 and 1:2 M ratio, before and after storage for 24 months at 25 °C 60% RH^{-1}

Differential Scanning Calorimetry

Usually, complexation of a drug with CDs produces a shifting of the melting point of the guest molecule. Then, thermal analysis is a useful tool to confirm such complexation. The DSC curves of the TCBZ and the corresponding complexes with HP-β-CD and Me-β-CD (1:1 and 1:2 ratios) are shown in Fig. 5. In agreement with a previous work, the thermogram of TCBZ exhibited a sharp endothermic peak at 177.83 °C, indicating its melting point (19). On the other hand, complexation of TCBZ with HP-β-CD at 1:1 ratio (Fig. 5a) showed an endothermic peak at 166.63 °C, confirming the existence of form II, as described by Tothadi *et al.* (45). As observed, the aged sample (Fig. 5c) exhibited a very similar peak (166.38 °C), suggesting that the drug did not undergo significant changes when complexed with HP-β-CD in an equimolar concentration. In contrast, at 1:2 ratio, the complete disappearance of the drug endothermal effect was instead observed in both fresh (Fig. 5b) and aged (Fig. 5d) complexes. In the case of the TCBZ:Me-β-CD complexes at 1:1 M ratio, it was found that the fresh sample (Fig. 5e) did not exhibit any peak of crystalline TCBZ suggesting the formation of an amorphous inclusion complex. The thermogram of the aged complex (Fig. 5g) indicated a peak at 165.90 °C, indicating that under these storage conditions, the drug might undergo gradual modification into a crystalline state. At 1:2 ratio, TCBZ:Me-β-CD complexes (Fig. 5f, h) did not show any endothermic peak of the drug, similarly to the behavior of the TCBZ:HP-β-CD complexes. In agreement with these results, it could be postulated that, an excess of the carrier (1:2 M ratio) may reduce the drug mobility avoiding the transition to a more stable crystalline state. In a similar report, Hirayama *et al.* described that chloramphenicol palmitate (CPP) was converted to an amorphous complex when formulated with HP-β-CD and no crystallization of CPP was detected after 2 months at 50 °C 50% RH^{-1} (48). Lately, Kimura *et al.* described the influence of aging on the crystallization and dissolution of tolbutamide complexed with HP-β-CD. It was observed that the carrier was able to modify the crystalline state of tolbutamide to an amorphous one, and also to keep the fast dissolution rate of the drug after storage for 1 week at 60 °C 75% RH^{-1} (49).

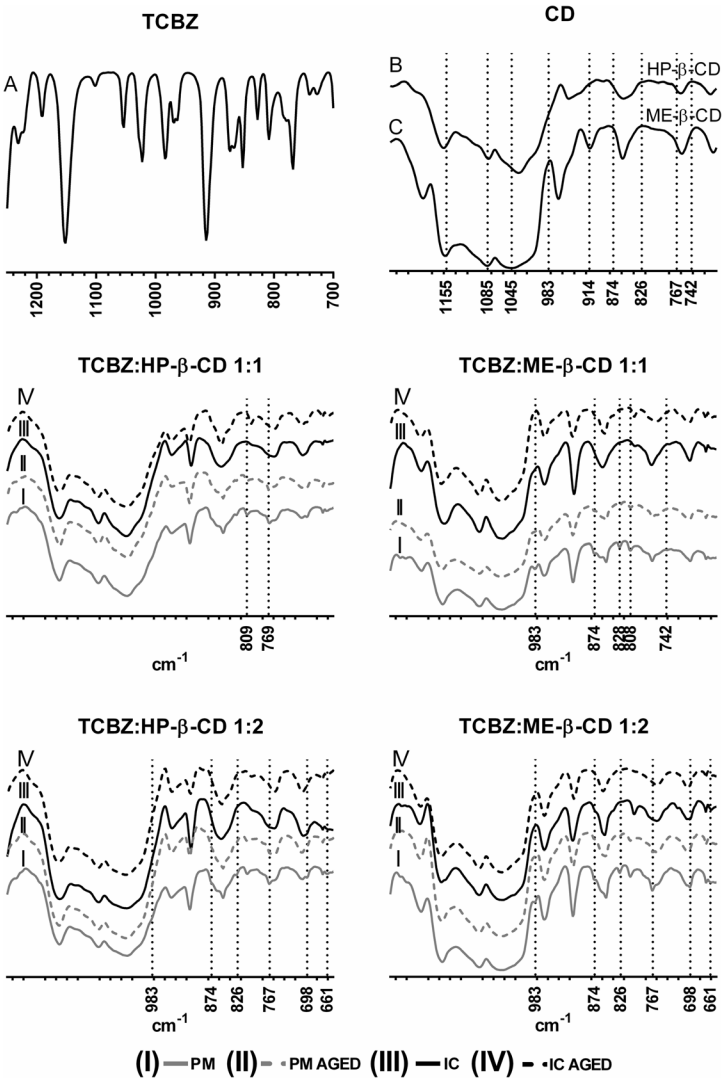


Fig. 4. FT-IR spectra of: **a** TCBZ, **b** HP-b-CD, and **c** Me-b-CD; TCBZ:CDs physical mixtures (PM) and TCBZ:CDs inclusion complexes (IC) at 1:1 and 1:2 M ratio, before and after storage for 24 months at 25 °C 60% RH^{-1}

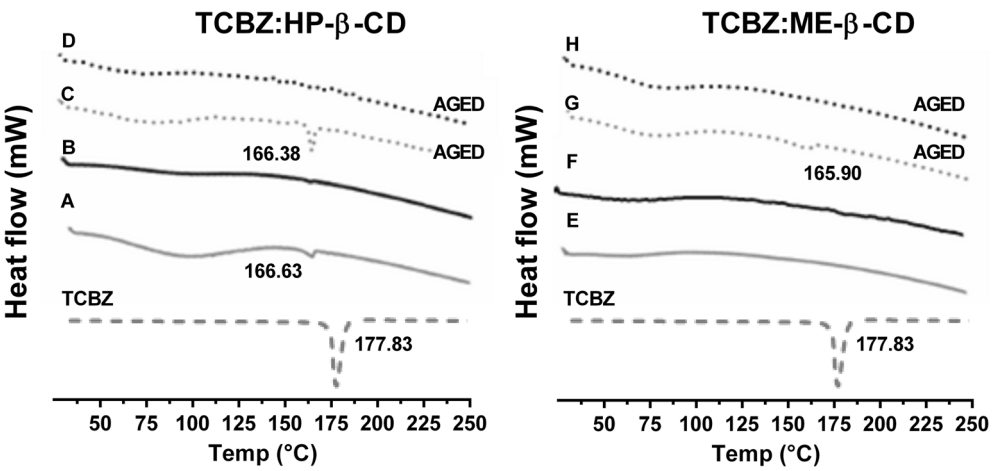


Fig. 5. Differential scanning thermograms for TCBZ; **a** TCBZ:HP-β-CD at 1:1 M ratio; **b** TCBZ:HP-β-CD at 1:2 M ratio; **c** TCBZ:HP-β-CD at 1:1 M ratio (aged); **d** TCBZ:HP-β-CD at 1:2 M ratio (aged); **e** TCBZ:Me-β-CD at 1:1 M ratio; **f** TCBZ:Me-β-CD at 1:2 M ratio; **g** TCBZ:Me-β-CD at 1:1 M ratio (aged); and **h** TCBZ:Me-β-CD at 1:2 M ratio (aged)

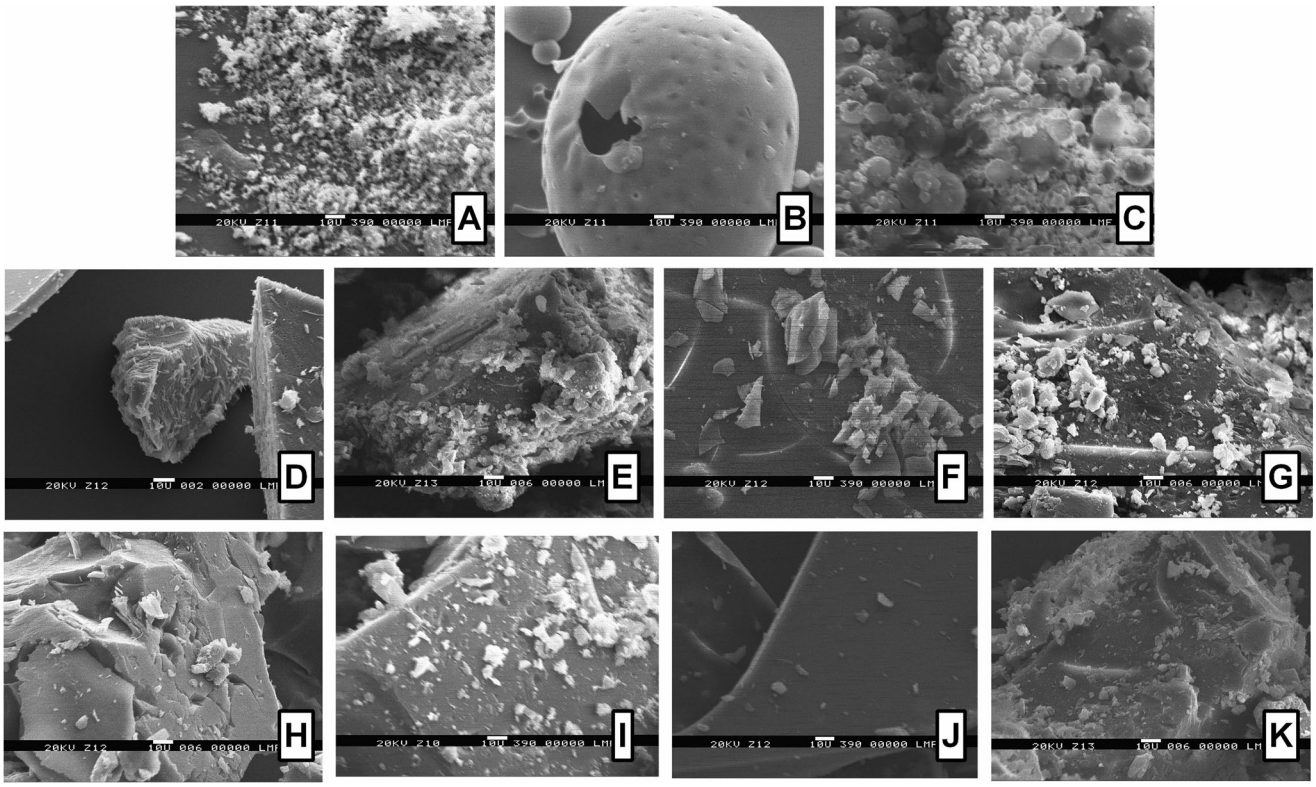


Fig. 6. Scanning electron micrographs of **a** TCBZ, **b** HP-β-CD, **c** Me-β-CD, **d** TCBZ:HP-β-CD at 1:1 M ratio, **e** TCBZ:HP-β-CD at 1:1 M ratio (aged), **f** TCBZ:Me-β-CD at 1:1 M ratio, **g** TCBZ:Me-β-CD at 1:1 M ratio (aged), **h** TCBZ:Me-β-CD at 1:2 M ratio, **f** TCBZ:Me-β-CD at 1:2 M ratio; **g** TCBZ:Me-β-CD at 1:1 M ratio (aged); and **h** TCBZ:Me-β-CD at 1:2 M ratio (aged)

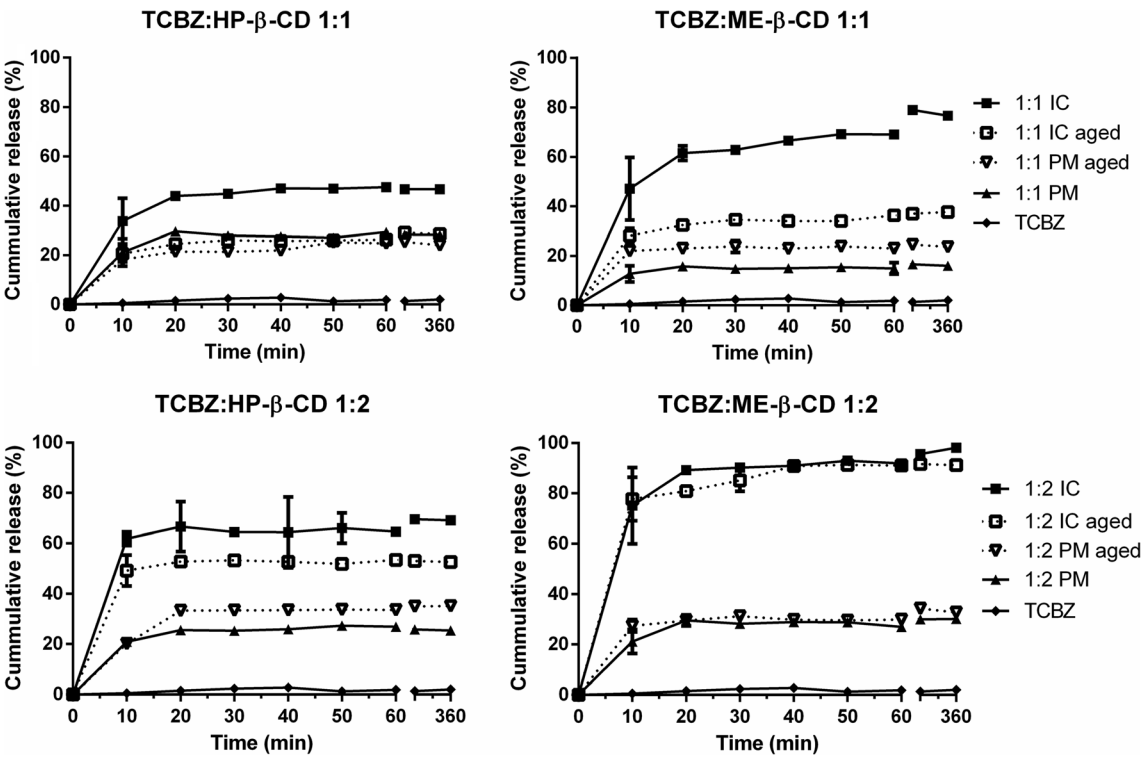


Fig. 7. Dissolution profiles of TCBZ:CD physical mixtures (PM) and TCBZ:CDs inclusion complexes (IC) at 1:1 and 1:2 M ratio

Table II. Dissolution efficiencies of TCBZ, TCBZ:CD physical mixtures (PM), and TCBZ:CD inclusion complexes (IC) at 10, 30, 60, and 90 min

System	Molar ratio	Treatment	Storage	Time (min)			
				10	30	60	90
TCBZ:HP-β-CD	1:1	PM	Fresh	10.58	21.59	24.68	26.13
			Aged	9.09	16.77	20.1	21.69
		IC	Fresh	16.91	33.4	40.07	42.46
			Aged	10.31	19.39	22.67	24
	1:2	PM	Fresh	10.43	19.69	23.05	24.23
			Aged	10.05	23.37	28.45	30.29
		IC	Fresh	30.95	53.6	59.28	61.28
			Aged	24.62	42.9	47.78	49.69
TCBZ:Me-β-CD	1:1	PM	Fresh	6.41	12	13.56	14.23
			Aged	11.01	19.01	21.23	21.87
		IC	Fresh	23.56	46.7	56.98	61.68
			Aged	14.05	25.95	30.25	32.31
	1:2	PM	Fresh	10.58	21.61	25.02	26.09
			Aged	13.7	24.16	27.05	28.23
		IC	Fresh	37.57	69.8	80.72	84.33
			Aged	38.86	67.03	78.57	82.53
TCBZ				0.28	1.09	1.89	2.16

Scanning Electron Microscopy

In order to investigate whether the formation of inclusion complexes of TCBZ could modify the surface and morphology of the particles and, therefore, influence the solubility and/or dissolution of the drug, SEM was performed for TCBZ, CD, and the prepared samples. As seen in Fig. 6, TCBZ (Fig. 6a) existed as irregular needle-type crystals while HP-β-CD and Me-β-CD and (Fig. 6b, c) were both observed as "shrinked" spheres, but the size of Me-β-CD (C) particles was notoriously smaller than the HP-β-CD (Fig. 6b) particles. In contrast, SEM images of the fresh-prepared complexes TCBZ:HP-β-CD at 1:1 and 1:2 M ratio (Fig. 6d, h) and TCBZ:Me-β-CD at 1:1 and 1:2 ratio (Fig. 6f, j) showed particles of irregular size being not possible to observe the original morphology of both TCBZ (Fig. 6a) and the CDs (Fig. 6b, c). Similar results were observed in the case of the stored complexes TCBZ:HP-β-CD at 1:1 and 1:2 M ratio (Fig. 6e, i) and TCBZ:Me-β-CD at 1:1 and 1:2 ratio (Fig. 6g, k). These changes in morphology and surface may effectively result in improved dissolution of TCBZ from the prepared complexes compared with the raw drug, as seen in Fig. 6.

Dissolution Studies

Although the solubility studies (Fig. 2) suggest that TCBZ solubility increase as a linear function of both HP-β-CD and Me-β-CD concentrations, which indicate the complex formation 1:1 drug:CD molar ratio, herein the complexes were formulated at 1:1 and 1:2 M ratio to investigate whether an excess of the carriers may affect the drug dissolution rate by the formation of both inclusion and noninclusion complexes (50). The influence of storage conditions (24 months at 25 °C 60% RH^{-1}) was also evaluated. Thus, the dissolution behavior of raw TCBZ and from the TCBZ:HP-β-CD and TCBZ:Me-β-CD (1:1 and 1:2 ratio) samples was evaluated before and after storage. As observed in Fig. 7, all the formulated samples exhibited a faster dissolution than the raw drug. In addition, TCBZ complexes showed faster drug release than that of the PMs. As expected, raw TCBZ presented a very low dissolution rate, since after 6 h only 2% of drug was in solution. Regarding the PMs, it was found that both fresh and stored TCBZ:HP-β-CD (1:1 and 1:2) showed around 25–30% drug release. These results suggested that a physical mixing of both components may improve the drug dissolution, probably due to the well-known solubilizing and wettability properties of CDs (51). A similar trend was observed in the case of TCBZ:Me-β-CD (1:1 and 1:2) where drug release was found to be around 20% in all samples. The analysis of TCBZ:HP-β-CD (1:1) complexes showed that fresh sample released more than 40% of drug while the aged one released around 25%. On the other hand, dissolution profiles of TCBZ:HP-β-CD (1:2) fresh and aged complexes indicated a drug release of 65 and 55%, respectively. Following the results obtained by evaluating these complexes, it is postulated that the decreased dissolution of TCBZ from aged samples could be due to the formation of the corresponding polymorph at 166.38 °C as seen in Fig. 4. On the other hand, fresh and aged TCBZ:Me-β-CD (1:1) showed 80 and 40% drug dissolution, respectively. This important difference could be due to a recrystallization of TCBZ after 24 months, and it is in agreement with the XRD diffractogram where a significant reduction of crystallinity is observed. In addition, a new small peak at 165.90 °C is seen by DSC in the aged complex, confirming the modification of the crystal properties of TCBZ, after storage, when complexed with Me-β-CD. In contrast, TCBZ:Me-β-CD (1:2) complexes were more efficient in terms of dissolution as compared with the other inclusion complexes. Both fresh and aged samples showed a more than 90% drug dissolution. Such similarities in the drug dissolution rate from the Me-β-CD complexes, might be due to the presence of amorphous form of TCBZ

before and after storage as seen in the X-ray diffraction studies (Fig. 2). The DE is a suitable parameter to compare and optimize the *in vitro* dissolution of different pharmaceutical formulations and also to analyze their *in vitro* biopharmaceutical performance (52). In addition, DE% is a useful tool to analyze the drug release data into a single table allowing, as a consequence, fast comparison between several formulations. Thus, in this study, DE values for both newly prepared and aged TCBZ:CDs PM and IC, at 10, 30, 60, and 90 min, were calculated. As seen in Table II, the results of the DE of all the samples are consistent with the dissolution profiles of the corresponding samples shown in Fig. 6. It is interesting to note that nonstoichiometric drug/CD complexes have been reported to increase the drug release as compared with the stoichiometric ones (53). Thus, it has been postulated that an excess of CD may disperse the lipophilic guest in a more efficient manner in the dissolution media and/or to improve the inclusion phenomena leading, as a consequence, to improved dissolution patterns (54). Also, the well-known wetting effect of the carrier should be considered in this case. On the other hand, it should be mention that the opposite effect was described by Semalty *et al.* (55). In this case, Racecadotril, a class II drug, complexed with β-CD at 1:1 M ratio showed a higher dissolution rate than the corresponding complex prepared at 1:2 M ratio, although no hypothesis was provided to justify such results.

CONCLUSION

To date, this is the first report on the dissolution behavior and solid-state analysis of TCBZ when complexed with CDs at different ratios before and after storage. As demonstrated by XRD, FT-IR, and DSC, the interaction of TCBZ with both HP-β-CD and Me-β-CD confirmed the formation of inclusion complexes. In particular, in the TCBZ-HP-β-CD (1:1) complex, the presence of the polymorphic form II was observed for the first time. The phase solubility data suggest a 1:1 complex formation with such cyclodextrins. All PM and inclusion complexes exhibited an increase in TCBZ dissolution as compared with the raw drug. In addition, nonstoichiometric complexes (1:2) demonstrated to be more effective than stoichiometric complexes (1:1) to improve drug dissolution. The amorphous character of the 1:2 complexes was maintained before and after storage and such data correlated well with the corresponding dissolution studies. Thus, these findings exhibit relevant pharmaceutical potential in view of developing a novel TCBZ delivery system.

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