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Interaction of Native Cyclodextrins and Their Hydroxypropylated Derivatives with Carbamazepine in Aqueous Solution. Evaluation of Inclusion Complexes and Aggregates Formation

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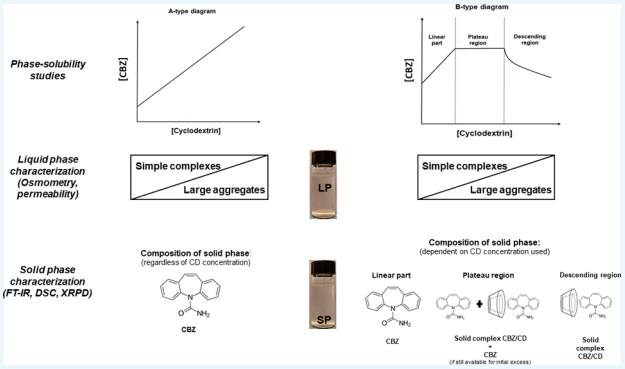
Supporting Information

ABSTRACT: A detailed comprehensive study on how the formation of soluble and insoluble carbamazepine/cyclodextrins (CBZ/CD) complexes (with consequent changes in the solid-phase composition) depends on the CD structure is not yet available. Moreover, the study of possible influence of this drug on the tendency of CDs and their complexes to self-aggregate is still lacking. Phase-solubility studies demonstrated that CDs and CBZ form a range of soluble (A_L-type: αCD, βCD, and hydroxypropylated CDs) and insoluble (B_S-type: γCD) complexes depending on CD used. HPβCD proved to be the best CD solubilizer for CBZ forming the most stable complex with highest apparent solubility, whereas γCD was shown to be the best native CD. For the native CDs, CBZ solubilization increases with increasing CD cavity diameter (αCD ≪ βCD < γCD). Solid phases collected from phase-solubility studies were characterized by Fourier-transformed infrared spectroscopy, differential scanning calorimetry, and X-ray powder diffraction to elucidate their composition and crystalline structure. They provided similar conclusions being overall supportive of phase-solubility, osmolality, and permeation studies results. Solid CBZ was the only detected component for A_L-type profiles over the CD concentration range studied, whereas precipitation of poorly soluble CBZ/γCD complexes (B_S-type) was observed (i.e., at and beyond plateau region). Osmometry and permeation studies were applied to evaluate the effect of CBZ on the aggregate formation and also to elucidate their influence on CD complex solubility and permeation profile. Permeation method was shown to be the most effective method to detect and evaluate aggregate formation in aqueous γCD and HPβCD solutions containing CBZ. CBZ did not affect the HPβCD tendency to self-aggregate but CBZ did modify the aggregation behavior of γCD decreasing the apparent critical aggregation concentration value from 4.2% (w/v) (in pure aqueous γCD solution) to 2.5% (w/v) (when CBZ was present).



1. INTRODUCTION

Carbamazepine (CBZ), 5*H*-dibenz[*b*,*f*]azepine-5-carboxamide, is the most widely used anticonvulsant drug for the treatment of psychomotor epilepsy. Although effective, CBZ constitutes a challenge for pharmaceutical formulators because of its low water solubility (approximately <200 μg/mL), slow dissolution rate, and polymorphism.[1−5] Four different anhydrous forms, and a dihydrate has been described in the literature, where polymorph form I and form III are the most frequently encountered.[6−8]

Although the different polymorphic forms of CBZ may exhibit different dissolution rates and bioavailabilities, selection of one polymorphic form over the others in a formulation may not be a viable route for improving the bioavailability of the drug. Other methods are needed to overcome the formulation limitations of CBZ (polymorphism and poor dissolution rate), one of which is the use of cyclodextrins (CD).[9−13] These pharmacopoeial approved excipients are very useful and used in several commercialized products (pharmaceutical, chemical, food, cosmetic, etc.) because of their safe toxicological profile and ability to form water-soluble complexes.[12,14−18]

In aqueous media, CDs can form inclusion complexes with a lipophilic molecule or part of a larger molecule or through formation of noninclusion complexes.[9,17,19] Solubilization of drugs, through a micelle-like mechanism, may also occur through formation of CD and/or drug/CD complex aggregates.[14,20] This ability of CDs to self-aggregate needs to be controlled by pharmaceutical formulators, as it sometimes needs to be prevented (can cause formulation instability provoking therapeutical and economical losses) but may also be advantageous for creation of novel drug delivery systems because of its simplicity and versatility.[12]

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Several studies of the interaction between CBZ and αCD,[21] βCD,[1−3,5,21−23] γCD,[13] HPβCD,[13,19,21,24−26] HEβCD,[13] DMβCD,[13] SBEβCD,[4,27] and HPγCD[13] have already been published. However, a systematic, detailed, and comprehensive study on the dependence of CD type on the formation of soluble and insoluble complexes is not yet available. The majority of the studies mentioned above utilize classic phase-solubility studies to characterize the potential of different CD types to increase the apparent solubility of CBZ by focusing on the increase of CBZ concentration in solution dependent on initial CD concentration. The composition and changes in composition of the solid phase are, as for the majority of studies on drug/CD complexes by phase-solubility experiments, fully ignored. Moreover, changes in the composition of the solid phase with respect to CD concentration as a consequence of possible formation of poorly soluble CD/drug complexes have also not been studied. Furthermore, none of the previous publications on this topic have focused on and described the possible influence of CBZ on the self-aggregation behavior of these CDs and their complexes. The aim of the present study is to provide a better understanding of the interaction of CBZ with a range of relevant CDs (varying cavity sizes: αCD, βCD, and γCD; and by comparing native and hydroxypropylated CDs) on the basis of phase-solubility studies including characterization of changes in the composition of the solid phase, changes in the concentration of CD in solution as compared to the initial concentration, and the formation of aggregates. To elucidate the composition and crystalline structure of the solid phases, we have characterized the solid phases obtained from the phase-solubility studies by Fourier transform infrared spectroscopy (FTIR), differential scanning calorimetry (DSC), and X-ray powder diffraction (XRPD), some of which are reported in the Supporting Information. The liquid phases were analyzed using permeation and osmolality methods for the determination of the influences of CBZ on the flux profile and apparent critical aggregation concentration (cac) of the CDs.

2. RESULTS AND DISCUSSION

In the first part of this study, we attempt to correlate the type of CBZ/CDs phase-solubility diagrams with the composition of the obtained solid phases and elucidate the importance of CD type on complexation and solubilization of these systems. To get insight into the nature of the solid phase, we have used diverse analytical techniques as previously mentioned. For these tests, A-type phase-solubility profiles were tested at the highest CD concentration used, whereas B-type phase-solubility profiles were at least tested at two concentrations (i.e., before and after the plateau region).

In the second part of this study, we aim to understand and evaluate not only if/how CBZ can affect the flux and apparent tendency of studied CDs to self-aggregate (comparing apparent cac values) when associated in inclusion/non-inclusion complexes but also how the CDs complexes modulate CBZ permeability through a semi-permeable membrane. This novel insight was mainly possible because of the performance of osmometry and permeation studies. Another novelty of this work compared with most publications in this field (where presented graphs and values are based on theoretical CD concentrations) was the use of Corona detector that allowed us to quantify the actual concentration of CDs and drug (simultaneously) present in liquid phases.

2.1. Phase-Solubility Studies. Usually the apparent water solubility of drug is enhanced by the formation of drug/CD complexes. To evaluate the interaction between CDs and CBZ, phase-solubility diagrams were made by plotting the concentration of CBZ brought into solution versus the actual (as opposed to added) CD concentration (Figures 1 and 2).

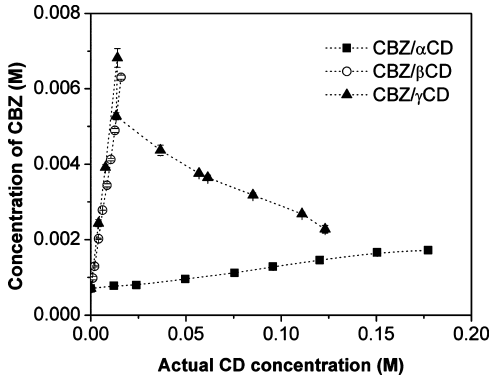

Figure 1. Phase-solubility profile describing solubility of CBZ in aqueous αCD, βCD, and γCD solutions at 25 °C. Symbols represent mean ± SD (n = 3).

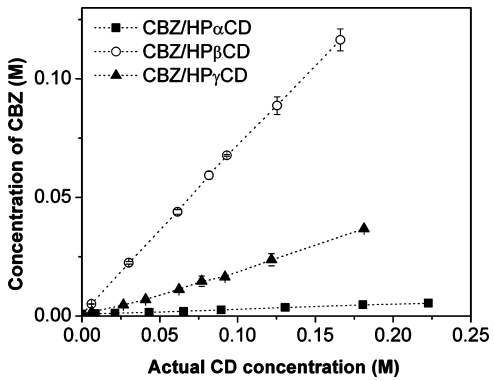

Figure 2. Obtained phase-solubility profiles for HP-CDs with CBZ (25 °C). Symbols represent mean ± SD (n = 3).

Slopes were calculated by plotting all concentration range in case of A-type diagrams or just using the initial linear portion (for B-type diagrams). Using the apparent intrinsic solubility (S_0) of CBZ and determined slopes, we were able to determine apparent stability constants ($K_{1:1}$) and the complexation efficiencies (CE). Also, the maximum increase (in percentage) in apparent solubility of CBZ per mM of CD (IS) was calculated (Table 1).

It has previously been described in the literature that native CDs have low aqueous solubility compared with their hydroxypropylated derivatives and that the native CDs are more prone to self-assembly and aggregate formation. This phenomenon can contribute for the definition of phase-solubility profiles of many drugs.[28,29]

2.1.1. Native CDs. Hydroxypropylated CD derivatives (i.e., HPαCD, HPβCD, and HPγCD) are obtained by random substitution that transforms the crystalline native CDs into amorphous mixtures of large number of isomers.[31,32] Thus, HPαCD, HPβCD, and HPγCD and their complexes are unable to form crystals in aqueous solutions and, because of their enhanced solubility, generally display A_L-type phase solubility diagrams. On the other hand, native CDs and their complexes

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Table 1. Type of Profile, Apparent Stability Constant ($K_{1:1}$), CE and Maximum Increase (in Percentage) in Apparent Solubility of CBZ per mM of CD (IS) in the Different Aqueous CBZ/CD Complex Solutions at 25 °C[a]

	carbamazepine (CBZ)				
CD	type[b]	slope	$K_{1:1}$ (M^{-1})	CE	IS (%)
αCD	A_L	0.006	8.9	0.006	0.97
βCD	A_L	0.348	840.5	0.53	56.4
γCD	B_S	0.441	1245.1	0.79	69.9
HPαCD	A_L	0.022	31.4	0.02	3.4
HPβCD	A_L	0.699	3299.5	2.33	110.1
HPγCD	A_L	0.189	369.0	0.23	31.5

[a]Phase-solubility diagrams built with actual CD concentrations instead of theoretical CD concentrations only showed some differences for the B-type diagrams (i.e., beyond the plateau region). As their initial linear part (i.e., before the plateau region) showed negligible changes, Higuchi−Connors classification could still be used. [b]A_L: linear phase-solubility diagram and B_S: phase-solubility diagram with linear initial increase, a plateau and a decreasing terminal solubility.[30]

can form crystalline solid state and, thus, display limited solubility in water that frequently leads to B-type phase-solubility profiles. However, in case of CBZ, only γCD presented the B_S-type profile while αCD and βCD displayed A_L-type (Table 1). We believe that for βCD, the A-type phase-solubility diagram is only observed because of the poor solubility of βCD (high enough βCD concentrations to observe B_S-type diagrams are not possible to achieve).

Figure 1 shows the phase-solubility profile for native CDs with CBZ. Combining the analysis of this figure with results of Table 1, it is possible to see that αCD and CBZ did not have a good interaction although it displayed A_L phase-solubility profile. $K_{1:1}$ and CE values (8.9 M^{-1} and 0.006, respectively) are extremely small pointing to the fact that αCD cavity is too small to host/interact with any part of CBZ.

As already mentioned, because of low aqueous solubility, βCD displayed an A_L phase-solubility profile with CBZ (Figure 1). Apparent solubility of formed complex increased linearly with increasing βCD concentration. Complexation of CBZ by βCD was possible and more effective when compared to αCD as all parameters described in Table 1 increased significantly.

Generally, γCD displays B_S-type profiles with guests and CBZ was not an exception. In Figure 1, a typical B_S-profile for γCD with characteristic regions is presented: First, a linear region, where the increase of CD concentration leads to the formation of water-soluble CBZ/γCD complexes that, consequently, increase CBZ total solubility. Thereafter a plateau region, where the maximum CBZ solubility is achieved, was expected. This region is not apparent from the data obtained. Finally, the descendent part is achieved and it is characterized by the decrease of apparent CBZ solubility due to formation of insoluble CBZ/γCD complexes. This happens because all the solid CBZ has been brought into solution or precipitated as CD complex and that the increase in γCD concentration will promote precipitation of the complex.

Overall, all calculated parameters presented in Table 1 (slope, $K_{1:1}$, CE, and IS) increased with increasing CD cavity size (αCD < βCD < γCD). Concerning the linear part of the phase-solubility diagrams, γCD registered the highest value for CE and IS of the native CDs showing that this was the native CD that interacted the strongest (i.e., formed most stable complex) and brought the most CBZ into solution.

All registered slopes were smaller than unity which suggests the formation of simple 1:1 CBZ/native CD complexes in accordance with literature.[3,13,21,22,24]

2.1.2. Hydroxypropylated Cyclodextrins. The phase-solubility diagrams obtained for the hydroxypropylated CDs (HP-CDs) also gave straight lines characteristic for A_L-type profiles (Figure 2).

These modified CDs were capable of solubilizing CBZ and to increase its apparent solubility with increasing CD concentration linearly. Similarly to αCD, HPαCD was also the least efficient CD of this group to bring CBZ into solution. The smaller cavity size probably hinders the interaction as revealed by the calculated parameters (i.e., $K_{1:1}$ = 31.4 M^{-1}; CE = 0.022).

On the basis of calculated parameters ($K_{1:1}$, CE, and IS), it is possible to see that HPβCD was overall the best CD tested because it formed the most stable complex and resulted in the largest increase in CBZ solubility (Figure 2 and Table 1).

The observed slope values were smaller than unity, which is an indication that complex formation follows a simple 1:1 stoichiometry also in the case of CBZ/HP-CD systems. Several authors using phase-solubility studies in combination with other analytical methods (e.g., FTIR, DSC, and XRPD) have also reported similar data. They also found evidence of simple inclusion complex formation (1:1 complexes) after interaction of CBZ with HPβCD[13,19,21,24−26,33] and HPγCD,[13,33] although always presenting smaller apparent $K_{1:1}$ values compared with those presented here. However, these discrepancies may be due to the use of a different type of HPβCD compared with the other studies (e.g., different manufactures of HPβCD producing products with varying DS).

Contrary to the native CDs, analysis of the slope $K_{1:1}$, CE, IS for CBZ/HP-CDs (Table 1) does not suggest direct influence of CD central cavity diameter on the increase of CBZ solubilization/complexation.

2.2. Characterization of Solid-Phase Compositions. The composition of solid phases resulting from the phase-solubility studies (Section 2.1) was determined by FTIR spectroscopy, DSC, and XRPD.

All techniques provided similar conclusions (see the Supporting Information), and results were supportive of phase-solubility experiments. The composition of the solid phase can be predicted from the phase-solubility diagram type and possibly by CD aggregation process (Table 2). This basically originates form the inherent ability of native CD to

Table 2. Variation of Solid-Phase Composition with Phase-Solubility Type Profile and CD Concentration

phase-solubility profile	solid phase's composition
A-type	highly soluble complexes remaining in solution.
	only excess of pure drug can be detected in solid phase.
B-type	content of solid phase will vary with CD concentration and surplus amount of model drug.
	region before plateau ≫ only pure model drug.
	plateau region ≫ a mixture of pure drug and insoluble complexes will be found.
	post plateau ≫ will mostly consist on solid complex, although it is not possible to exclude the presence of pure drug (depending on the initial excess of drug added and availability of CD in liquid phase at that stage).

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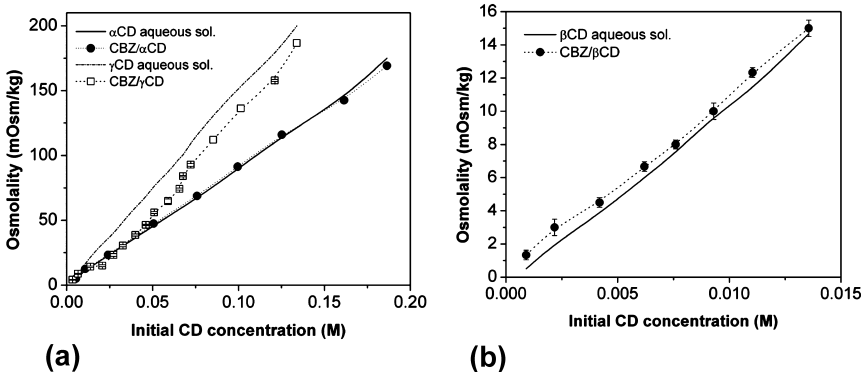

Figure 3. (a) Changes of total osmolality for the prepared CBZ/αCD and CBZ/γCD systems during phase-solubility experiments (25 °C). (b) Changes of total osmolality for the CBZ/βCD systems during phase-solubility experiments (25 °C). Symbols represent mean ± SD (n = 3).

form insoluble complexes (resulting in B-type phase solubility diagrams), a trait not found for typical randomly modified CD's (e.g., commercially available hydroxypropyl derivatives). Here, their inability to form insoluble complexes most often results in A-type diagrams in the concentration ranges studied.

2.3. Osmolality Measurements. Osmometry is a colligative property that indicates the number of all osmotically active molecules dissolved in the solvent and in our previous research proved to be quite useful to detect native CDs aggregates.[34]

In an attempt to detect/quantify CBZ/CD inclusion complexes aggregates and also tracing possible changes in the apparent solubility of the CD complexes as a consequence of interaction with soluble CBZ, the osmometry method was applied to liquid phases from the phase-solubility experiment after careful separation of the liquid and solid phases.

The osmolality of freshly prepared CD aqueous solutions (represented by lines) and CD solutions saturated with CBZ (represented by symbols with lines) were plotted against CD concentrations. We expect that total osmolality will remain constant or become slightly higher in case of the formation of soluble complexes. Total osmolality will be equal to osmolality of CD plus the one obtained from increased apparent solubility of CBZ (as CD is present in higher amount, usually this possible osmolality increase is only visible for βCD complexes). On the other hand, we will observe a decrease in total osmolality in the case of systems that displayed phase-solubility B-type, as from some region (beyond plateau) precipitation of solid complex will decrease the particle concentration in the solution.

2.3.1. Native CDs. Different trends in osmolality behavior were registered for native CDs after interaction with CBZ, depending on the sample phase-solubility type (Figure 3).

For CBZ/αCD liquid phases, almost no changes were observed in osmolality from samples saturated with CBZ (Figure 3a). This is in accordance with phase-solubility results as the presented osmolality plot is typical from A_L-type profiles.

In the case of CBZ/βCD, the presence of CBZ promotes a small constant increase of the osmolality of the CD solutions throughout the concentration range (Figure 3b).

Commonly, samples containing γCD show negative deviations of osmolality from stock solutions trend, caused by a decrease in particle concentration due to the precipitation of poorly soluble CBZ/γCD complexes at a certain CD concentration (Figure 3a). The B_S-type profile nature of this system justifies the appearance of CBZ/γCD osmolality graphic that can be divided into three characteristic regions. As saturation concentration for the complex is achieved with relatively low γCD concentrations, the two first regions are relatively short. First, we observe the linear region (until ∼0.006 M) where the CBZ/γCD complex contributes to the increase of system osmolality because the osmolality of the solution containing both CBZ and γCD is higher than that observed for the γCD stock solution alone. This effect will continue until the crossing point with the CD curve (second region represented by a small plateau from ∼0.006 to 0.014 M) where the poorly soluble CBZ/γCD complex starts to form and overall osmolality will remain almost unalterable. Then, we enter on the last region for this system (beyond plateau region, from ∼0.014 M), where precipitation of poorly soluble complex stops because of unavailability of solid CBZ, and adding more γCD will only increase the amount of free γCD in solution. Because of this, the total osmolality of the system starts to increase again, however, never reaching γCD stock solution values (dot line in Figure 3a).

2.3.2. Hydroxypropylated Cyclodextrins. Similarly, to the CBZ/αCD and CBZ/βCD systems (A_L-type), also the saturation of HP-CDs with CBZ did not provide any significant change on system total osmolality (Figure 4). It is typical for A-type profiles that throughout the CD concentration range, the amount of osmotically active particles remains the same or slightly higher in comparison with the initial CD stock solution. The described behavior observed in Figure 4 excludes the possibility of formation and precipitation of CBZ/HP-CDs inclusion complexes.

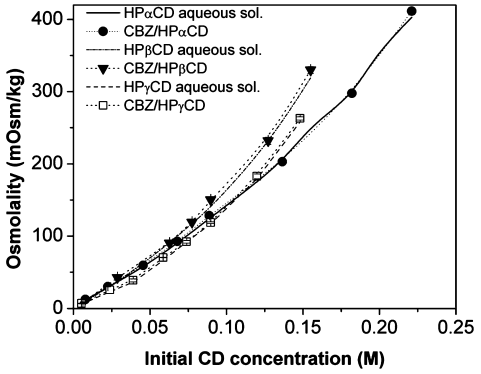

Figure 4. Total osmolality changes for of the prepared CBZ/HP-CD systems during phase-solubility experiments (25 °C). Symbols represent mean ± SD (n = 3).

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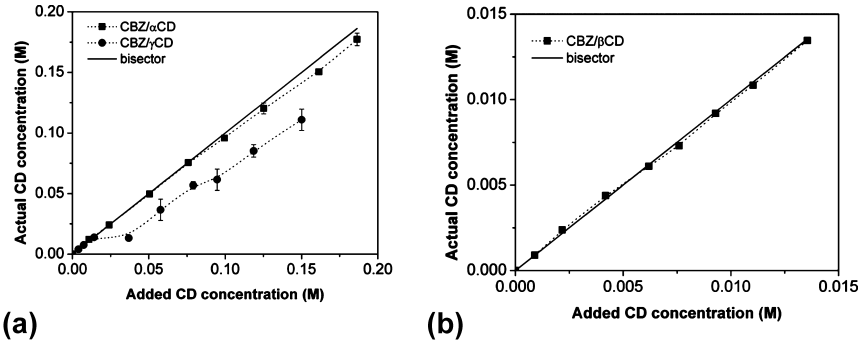

Figure 5. Illustration of α- and γCD (a) and βCD (b) solubility in the prepared CBZ/CD systems. Symbols represent mean ± SD (n = 3).

Altogether, these observations corroborate phase-solubility results for HP-CDs, where they displayed A_L-type.

Osmometry proved to be a simple and reliable method to determine phase-solubility profiles of the studied systems.

However and contrary to what we described for native CD aqueous solutions,[34] this method was not capable of detecting CD aggregate formation and determine the possible modulation of these inclusion complexes on the self-assembly process of the CDs. The precipitation of inclusion complexes (that occurs for B-type profiles) and the increase of the systems osmolality due to increase of CBZ concentration makes it difficult to interpret deviations that occur in total osmolality of these systems (e.g., osmolality can decrease due to precipitation of components, presence of aggregates, etc.).

2.4. Solubility of Native and HP Derivatives in the CBZ Media. To find a plausible justification for the osmolality depression observed in previous chapter, the possible effect of CBZ on CD complexes solubility was accessed. Graphical representations of added CD concentration against actual CD concentration (CD concentration in liquid phase after equilibrium) during phase-solubility studies are provided in Figures 5 and 6. Bisectors (described by solid lines) representing the theoretical situation where added CD concentrations equals that in solution were also included in the figures.

For CBZ/αCD and CBZ/βCD, no significant difference is observed between added αCD/βCD concentration and the concentration at the end of experiment (Figure 5).

A different behavior was observed for γCD where no changes in added and actual CD concentration are observed initially in the linear region diverting from the bisector after approximately 0.014 M γCD (Figure 5a). After saturation concentration of CBZ/γCD inclusion complex is achieved (matching with the point of diversion from bisector), the plateau region is reached and precipitation of the complex (consequently also CD) takes place. When solid CBZ is no longer available in the system, the additional amount of CD provided will lead to its accumulation in liquid phase. This phenomenon explains the increase in actual γCD concentration visible in Figure 5a after the plateau region (from ~0.037 M).

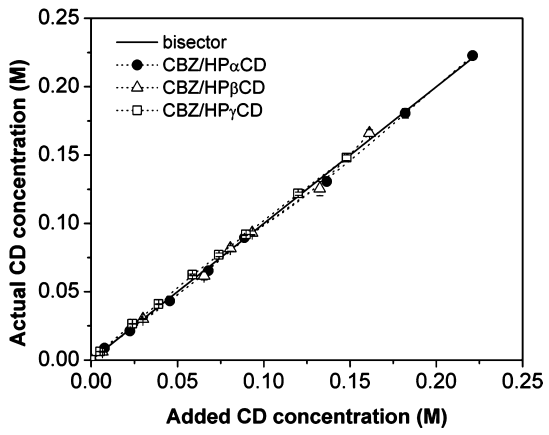

Figure 6. Illustration of HP-CDs solubility in the prepared CBZ/HP-CD systems. Symbols represent mean ± SD (n = 3).

Figure 6 shows that CBZ, as expected by A_L phase-solubility profile, did not have any influence and changed the solubility of HP-CDs over the phase-solubility experiment. This can be explained by the formation of complexes with high aqueous solubility.

2.5. Permeation Studies. For these experiments, two representative CBZ/CD systems were selected: native γCD and HPβCD. These represent two of the most promising candidates for formulation of CBZ as they display the highest CE, apparent $K_{1:1}$ and IS of the studied CDs. Furthermore, they represent two distinctly different phase-solubility profiles (B_S and A_L-type, respectively) and are used in the pharmaceutical field. Thus, it is important to achieve more knowledge on the possible influence that guests and inclusion complexes can have on the self-assembly behavior of these CDs. Knowing more about how guests with different physicochemical properties can modulate the solubility and aggregation process of CDs complexes, we can try to disclose driving factors that can promote or prevent the formation of these structures.

This fits in one of the goals we set for this work, the detection and quantification of CD aggregation in the selected systems (through apparent cac values). From permeation studies, the apparent cac cannot be defined as the concentration where CDs start to assemble in larger structures (CD aggregates) but rather the concentration at which the size starts to be a hindering factor for free flow of CDs through the membrane (i.e., the aggregates size becomes greater than the molecular weight-cutoff (MWCO) pore size of the membrane tested and the cac determined is thus dependent on the choice of MWCO of the membrane).

2.5.1. Hydroxypropylated βCD. The potential effect of CBZ on HPβCD aggregation behavior was estimated through Franz diffusion cell permeability studies using liquid phases from phase-solubility studies. For this group of experiments, 3.5−5 kDa membrane was used and the flux of HPβCD from CBZ/HPβCD systems was determined. Afterward, the flux values were plotted versus HPβCD concentration and a tangent line

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to the flux curve was drawn to determine the concentration point from where the flux curve started to divert from linearity. This estimated value corresponds to the apparent cac value for this MWCO pore size membrane.

Sink conditions[35] were fulfilled for this set of experiments (both HPβCD and γCD) as donor phases did not register significant volume changes (were always less than 0.1 mL), and CD concentration in the end of permeability experiments (donor phase) was always higher than 90% of initial concentration.

In Figure 7, it is possible to observe the flux curves for HPβCD aqueous solutions (filled squares) and for HPβCD

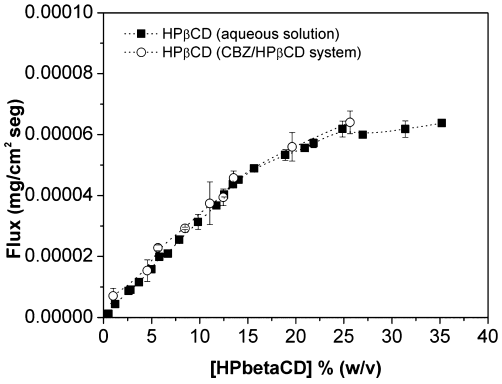

Figure 7. Flux profile of HPβCD from HPβCD aqueous solution (filled squares) and CBZ/HPβCD liquid phases (empty circles) through 3.5−5 kDa MWCO semipermeable membrane.

from CBZ/HPβCD liquid phases (open circles). The flux behavior of HPβCD aqueous solutions was previously studied by our group.[36] Apparent cac value for this system with 3.5−5 kDa MWCO membrane (the same as for the present study) was determined to be 11.8% (w/v). This means that until the apparent cac value HPβCD and any formed aggregates permeated freely through the membrane to the receptor phase because they did not have a particle size hindering their diffusion through the membrane pores. Knowing that the molecular weight of HPβCD is approximately 1.36 kDa, we could estimate from the apparent cac that the aggregates consisted of approximately 3−4 HPβCD monomers at cac.

It is understandable from Figure 7 that no significant difference could be found between HPβCD flux curves from HPβCD solutions and HPβCD from CBZ/HPβCD. Apparent cac values (for 3.5−5 kDa) for the CBZ/HPβCD systems was estimated to ∼11.6% (w/v) which is similar to the one found for pure HPβCD solutions. The presence of CBZ did not affect or promote the formation of HPβCD aggregates with larger sizes than the one that are naturally formed in HPβCD aqueous solutions. Altogether, these results suggest that CBZ did not affect the self-assembly process of HPβCD.

2.5.2. Native γCD. Similar to what is described above, the flux curve of γCD from CBZ/γCD liquid phases was plotted against actual γCD concentration and compared with the flux curve from γCD aqueous solution (Figure 8). The apparent cac value (3.5−5 kDa MWCO) for both systems was calculated and used to study the possible effect of CBZ on natural aggregation of γCD.

Small but significant differences are noticeable when the flux curve for γCD in aqueous solution (filled squares) is compared with the one from γCD from CBZ/γCD samples (Figure 8).

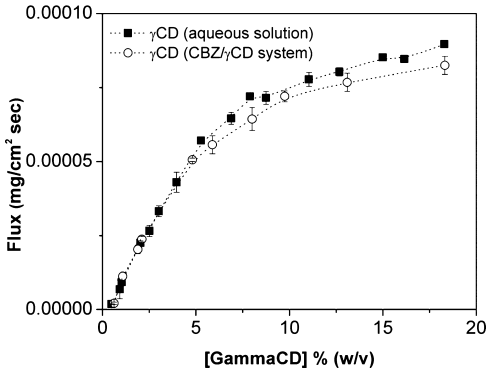

Figure 8. Flux profile of γCD in aqueous γCD solution (filled squares) and γCD from CBZ/γCD liquid phases (empty circles) through 3.5−5 kDa MWCO semipermeable membrane.

γCD flux in aqueous solution increased linearly until a concentration of 4.2% (w/v) (apparent cac value for 3.5−5 kDa) after which a negative deviation probably due to the formation of aggregates and increased in size of aggregates is observed. Beyond this, γCD concentration aggregates are most likely assembled in sizes larger than the membrane pore size selected for this study, hindering the free flux of γCD from donor to receptor phase. We can estimate that after 4.2% (w/v) γCD (MW 1297 Da) solutions, we have aggregated populations of trimers and/or tetramers.

Regarding γCD from CBZ/γCD system, we can observe that flux values after a certain γCD concentration significantly dropped in comparison to γCD in aqueous solution. This observation was supported by a decrease in the apparent cac value which was estimated to 2.5% (w/v). Until this value, aggregates of γCD and CBZ/γCD complexes could be present in the solution with a maximum size of dimers or trimers which are not large enough to make size a permeation limiting factor. After 2.5% (w/v) of γCD, the flux deviated from linearity as CBZ started to promote the formation of larger structures with sizes larger (more than 3 monomers) than the membrane pore size used in this study (3.5−5 kDa).

The CBZ/γCD system displayed a B_S-type diagram in the phase-solubility studies, and it is interesting to note the apparent cac value (for 3.5−5 kDa) and the γCD concentration at which highest solubility of CBZ/γCD complex coincides. This suggests that the B_S-type profile and decrease in apparent CBZ solubility after the plateau region might be related to the aggregate formation of the complex that starts to precipitate out of solution as solid crystalline complexes after this concentration have been reached.

All the collected data strongly suggest that CBZ had an influence on the promotion of γCD aggregates formation. Similar results were reported in previous publication where indomethacin[37] and parabens[15] proved to influence γCD aggregation and modulate its permeation profile.

2.5.3. Influence of HPβCD and γCD on Permeation Behavior of CBZ. In previous chapters (2.5.1 and 2.5.2), we described the influence of CBZ on the permeability and aggregation profile of HPβCD and γCD complexes. In the present one, we want to investigate the opposite effect, that is, how these two CDs may affect the permeability of CBZ.

Both CDs were capable of forming inclusion complexes with CBZ and thus capable to increase CBZ flux when compared with CBZ alone (in aqueous solution). Figure 9 exemplifies the CBZ flux variation for CBZ/HPβCD samples (Figure 9a) and

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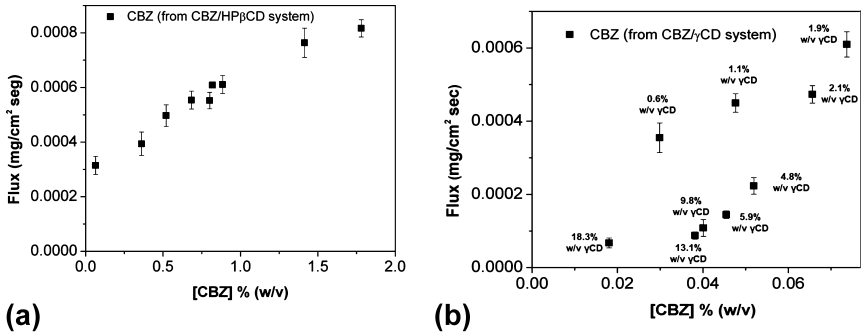

Figure 9. Flux profile of CBZ from CBZ/HPβCD liquid phases (a) and of CBZ from CBZ/γCD liquid phases (b) through 3.5−5 kDa MWCO semipermeable membrane.

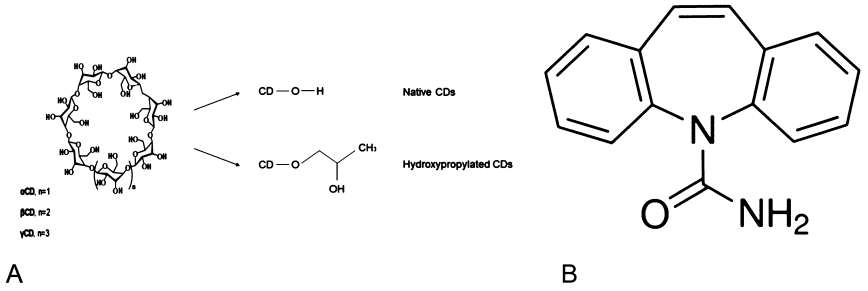

Figure 10. Schematic representation of native and hydroxypropylated CDs (A) and CBZ (B). Adapted from ref 39.

for CBZ/γCD samples (Figure 9b) versus its respective measured CBZ concentration.

Previously, we proved using permeation data that CBZ was not able to modify the natural HPβCD permeation profile and to promote any influence over HPβCD aggregation. Through evaluation of Figure 9a, we can see a linear increase of CBZ flux with CBZ concentration (following Fick's first law) promoted by the CBZ/HPβCD compared with CBZ SE. HPβCD is able to increase and facilitate the flux of CBZ molecules from donor to receptor chamber (higher amount of CBZ/HPβCD complexes led to increase of CBZ apparent solubility).

Contrarily, CBZ flux from complexed CBZ/γCD did not present a linear relation with γCD concentration (Figure 9b). Also, here a possible correlation of phase-solubility type profile (B_S-type) with the CBZ flux profile (from CBZ/γCD system) can be used to explain the peculiar shape presented. This flux curve starts with two points with relatively high flux values (0.03%/3.5 × 10^{-4} and 0.048%/4.5 × 10^{-4}) corresponding to the linear part of phase-solubility experiment for this system. The presence of increasing amount of soluble CBZ/γCD inclusion complexes with γCD concentration justifies the increase of CBZ apparent water solubility, as well as the observed flux linear increase. However, it stops as soon as saturation concentration of complex is achieved. This point is depicted in Figure 9b as the one presenting simultaneously highest flux and also highest CBZ concentration (0.073%/6.1 × 10^{-4}).

Afterward a region where CBZ concentration decreases (due to the precipitation of CBZ/γCD complexes) and simultaneously the flux (increasing presence of γCD aggregates) with increasing actual γCD concentration can be observed. The points analyzed corresponded already to plateau region and beyond. We know that γCD concentration in solution is stable along plateau part and slowly starts to increase after it (the amount of available γCD in the liquid phase at this stage will depend on the solid CBZ added at the beginning of experiment). If γCD would not affect permeability of CBZ (other than facilitate it by increasing its solubility), we should observe an overlapping with previously described CBZ flux points. This means that samples with same CBZ concentration should present same flux values, however, that did not happen because those samples have also different amounts of γCD which probably explains the peculiar shape flux curve registered.

From the analysis of these results, it is possible to suggest that when the γCD concentration is increased in solution, more and larger aggregates were formed. These superstructures might be the involved on the precipitation of inclusion complexes and by a decrease of CBZ in liquid phase as a consequence. Their presence in the donor phase might hamper the diffusion of matter from donor to receptor phase leading to the marked drop of CBZ flux shown in Figure 9b.

Our finding that formulations based on inclusion complexes of CBZ/γCD providing similar concentrations of CBZ (but using different amounts of γCD) can be produced with different permeability capacities can be an advantage to the pharmaceutical research field. It might be a useful tool especially for ocular or dermal delivery as different types of drug delivery systems can be produced just by using different concentrations of γCD (or other native CDs) in the formulation (Figure 10).

We can hypothesize that small amounts of γCD (until plateau region) will allow the production of formulations with fast permeation of components (mainly constitutes by monomers and small aggregates). On the other hand, formulating with higher γCD concentrations (over plateau region and beyond), γCD and CBZ/γCD complex aggregates with larger size and in higher number are formed. The inclusion complex permeability will be slowed down and, consequently, sustained-permeation delivery systems can be produced.

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3. CONCLUSIONS

Several interesting conclusions could be made from this experimental work. The phase-solubility data could be fitted to a model based on simple 1:1 complex formation suggesting, as previously presented by other authors, that CBZ formed 1:1 complexes with all tested CDs. This does not prove that 1:1 complexes are formed and are responsible for the appearance (linearity) of the phase-solubility curves. It only signifies that the data can be fitted to a model for complex formation based on a simple 1:1 stoichiometry. The lowest apparent solubility increase was obtained for αCD which almost did not interact with CBZ because of small cavity size (although it displayed A_L-type), and for γCD which formed complexes with limited water solubility (B_S-type), CD cavity size of the native CDs showed to be an important factor for the better solubilization of the drug, increasing with increasing diameter (αCD $\ll$ βCD < γCD).

Expectedly, hydroxypropylated CD derivatives also displayed A_L-type. Because of the larger applicable range of CD concentrations, the solubility enhancement achieved for CBZ can reach higher values than that of their correspondent parent CDs.

HPβCD was the best solubilizer agent for CBZ forming the complexes with highest solubility and was most stable ($K_{1:1}$ = 3296.3 M^{-1} and CE = 2.3). γCD proved to be the best option from native CDs to solubilize CBZ ($K_{1:1}$ = 928.9 M^{-1}; CE = 0.66 and IS = 69.9%/mM).

Overall the solid-state characterization techniques, as well as osmometry data, provided similar conclusions and were supportive of liquid phase results (phase-solubility, osmolality, and permeation studies). However, in this study, osmometry was not capable to reveal any possible influences of CBZ and its complexes on the aggregation behavior of the CDs (and consequently to determine apparent cac values). Nevertheless, osmometry is a fast and reliable method to corroborate or determine phase-solubility profiles.

Permeation studies have shown to be an extremely useful tool to study aggregation and permitted the calculation of apparent cac values for both HPβCD and γCD systems.

CBZ did not affect or promote the self-assembly process of HPβCD contrary to γCD where CBZ had a clear influence on the formation of its aggregates.

HPβCD had a clear influence on the CBZ flux, increasing linearly with CBZ concentration due to the increase of complexes formed facilitating permeation of CBZ through the membrane. For γCD, aggregates significantly changed the flux of CBZ through the semipermeable membranes because samples with similar CBZ concentration but with different concentrations of γCD present completely different permeation abilities as opposed to overlapping of flux curves (more and larger aggregates were formed when γCD concentration increased yielding lower permeability and consequently lower overall flux).

4. MATERIALS AND METHODS

4.1. Materials. The native CDs (αCD, βCD, and γCD) as well as 2-hydroxypropyl-βCD (HPβCD) DS 4.2 (MW 1380) were kindly provided by Janssen Pharmaceutica (Beerse, Belgium). 2-Hydroxypropyl-αCD (HPαCD) with degree of substitution 3.6 (MW 1180) and 2-hydroxylpropyl-γCD (HPγCD) DS 4.2 (MW 1540) were purchased from Wacker Chemie (Burghausen, Germany). CBZ was also kindly provided by Janssen Pharmaceutica (Beerse, Belgium). The solvent used for analysis (acetonitrile) was of high-performance liquid chromatography (HPLC) grade and obtained from Sigma-Aldrich (St. Louis, Missouri, USA). Milli-Q water (Millipore, Billerica, MA) was used to prepare CD solutions and mobile phases.

4.2. CD Solutions Preparation. Different aqueous CDs stock solutions (taking into account the intrinsic solubility of CD being tested) were prepared. To promote faster dissolution of the CDs, sonication at elevated temperature was used (60 °C/60 min) after which solutions were allowed to cool to room temperature. The test solutions were then prepared by dilution of these stock solutions. The CD concentration range depended on the specific CD.

4.3. Quantitative Determination of CD/CBZ. A reverse-phase ultra-HPLC (UHPLC) system from Dionex Softron GmbH (Germering, Germany) was used for the simultaneous determination of CBZ and CDs. The Ultimate 3000 series consisted of a LPG-3400SD pump with a built-in degasser, a WPS-3000 autosampler, a TCC-3100 column compartment, and a Corona ultra RS detector. Phenomenex Kinetex C18 150 × 4.60 mm 5 μm column with a matching HPLC Security Guard (Phenomenex, Cheshire, UK) was used. Acetonitrile and water (50:50) were the components of mobile phase. The flow rate was set to 1.0 mL/min, and temperature of the column was set to 30 °C. The injection volume was 10 μL. Chromatograms were evaluated using ChromeleonR version 7.2 SR4 (ThermoFisher Scientific, MA, USA).

4.4. Phase-Solubility Experiments. Phase-solubility profiles for the CBZ/CD systems were achieved using the isothermal saturation method. Solid CBZ was added in excess amount (that assure the formation of solid phase in equilibrium stage) to each vial together with 3 mL of CD solution of given concentration. This methodology was used for all six CDs tested and was performed in triplicate.

The formed suspensions were kept at 25 °C under constant stirring. After reaching equilibrium (48 h), the mixture was centrifuged for 10 min/3000 rpm using Rotina 35R (Hetich Zentrifugen, Germany). Liquid phases were filtered through a 0.45 μm Phenex-RC filter (Phenomenex, Cheshire, UK) and then diluted with Milli-Q water prior to UHPLC analysis. Solid phases were collected and dried in an oven at 35 °C for two days.

The Higuchi and Connors[30] method was used to classify the phase-solubility profiles. The apparent stability constant ($K_{1:1}$, eq 1) and the CE (eq 2) were determined from the slope of the linear phase-solubility diagrams plots of the total drug solubility ($[D_t]$) versus total concentration of CD in liquid phase ($[CD_{liq}]$) in moles per liter[26]

$$K_{1:1} = \frac{\text{slope}}{D_0(1 - \text{slope})} \tag{1}$$

$$\text{CE} = \frac{\text{slope}}{1 - \text{slope}} = \frac{[D_t]}{[CD_{liq}]} = K_{1:1} \times S_0 \tag{2}$$

where S_0 is the intrinsic solubility of the CBZ.

The maximum increase (in percentage) in apparent solubility of CBZ per mM of CD (increase in solubility, IS) for each CBZ/CD system was determined as follows (eq 3)

$$\text{IS} = \frac{[CBZ_{max}] - [CBZS_0]}{[CBZS_0] \times [CD_{max}]} \times 100 \tag{3}$$

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where CBZ_{max} corresponds to highest solubility of complex and CD_{max} corresponds to the actual concentration of CD needed to achieve it (measured in the liquid phase at equilibrium). This value was calculated and is valid for linear part of phase-solubility diagrams.

4.5. Osmolality Measurements. Osmolality measurements were used to evaluate the aggregation behavior of the CBZ/CD systems. To determine the osmolality of liquid phases, a freezing point osmometer OSMOMAT 30 (Gonotec GmbH, Germany) was used. Calibration was performed with three points: Milli-Q water and by saline standard of 300 or 400 mOsmol/kg NaCl/H_2O (KNAUER, Germany) depending on concentration range of analyzed samples. For this procedure, only 50 μL of the sample was required. Samples were measured immediately after filtration of liquid phases to avoid possible precipitation of components during storage.

4.6. Permeation Studies. Unjacketed Franz diffusion cells with diffusion area of 1.77 cm^2 (SES GmbH—Analyse systeme, Germany) were used to determine the possible influence of CBZ on γCD and HPβCD apparent cac values and the effect of these CDs on the capability of CBZ to penetrate semipermeable membranes.

12 mL of Milli-Q autoclaved water (to remove dissolved air) were used as receptor phase, while the donor phase consisted in 2 mL of different liquid phases (from phase-solubility experiments). In between the two compartments, a 3.5−5 kDa MWCO semipermeable cellulose ester membrane (Biotech CE, Spectrum Europe, Breda, NL) was placed. All permeability experiments were carried out at room temperature under continuous magnetic stirring (300 rpm) of receptor phase (donor phase was unstirred).

Sampling was initiated after 1 h for γCD and 0.25 h for HPβCD after which the amount of CBZ diffused into the receptor phase was above the quantification limit. Samples were withdrawn every 15 min hereafter at determined time points: 60−120 min for γCD and from 15 to 75 min for HPβCD, 150 μL of sample was collected from the receptor phase and immediately replaced by equal volume of Milli-Q water. UHPLC was used to simultaneously quantify CD and CBZ. Steady-state flux (J) of the CBZ/CD was calculated from the slope (dq/dt) of the linear regression relationship between time (t) and the amount of CBZ and CD in receptor phase (eq 4)

$$J = \frac{dq/dt}{A} = P_{app} \cdot C_d \quad (4)$$

where A is the diffusion area (1.77 cm^2), P_{app} is the apparent permeability, and C_d is the total CBZ/CD concentration in the donor phase.

To assure sink conditions, common guidelines were followed, where both volume change and final concentration of components in donor phases were assessed.[35,38]

The calculation of the apparent cac for 3.5−5 kDa (i.e., concentration from where aggregates size starts to be larger than the studied pore size, leading to a deviation of ideal flux of CDs through the membrane) was performed by drawing tangent lines in the flux graphics (starting at low concentrations). The deviation point from where the flux started to divert from linearity corresponded to apparent cac value.[34]

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsomega.8b02045.

Analysis of the solid CBZ/CD complexes by FTIR spectroscopy, DSC, and X-ray powder diffractometry (XRPD) (PDF)

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Notes

The authors declare no competing financial interest.

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