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ORIGINAL ARTICLE

Interaction of native CDs and their hydroxypropyl derivatives with parabens in aqueous solutions. Part 2: evaluation of paraben/cyclodextrin complex aggregation

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Abstract

Cyclodextrins (CDs) and their inclusion complexes are known to self-assemble in aqueous solutions to form aggregates and the physicochemical characteristics of guest molecules have been linked to the aggregate formation. A series of parabens was selected as model compounds due to their small size (aromatic ring fits in the cavity) and different side chain length. In Part 1 it was demonstrated that CDs and parabens form a range of soluble and insoluble complexes dependent on the type of CD (native or hydroxypropylated αCD, βCD or γCD) and the length of the alkyl residue of the parabens. Furthermore, phase-solubility studies suggested that higher order complexes (e.g., aggregates) were formed. Here we apply osmometry and permeation studies to evaluate if and how the alkyl chain length of the parabens influences the process of aggregate formation. Furthermore, the possible effect of CD aggregates on permeation profile of parabens is also elucidated. Changes in osmometry correlate with the type pf phase-solubility profile. For A_L-types total osmolality remained unchanged throughout experiment, while for B-types the osmolality of systems displayed significant changes mainly due to precipitation of poorly-soluble complexes. The permeation method is an effective and useful method to detect and evaluate self-assembly of CDs and to detect aggregate formation in aqueous γCD and HPβCD solutions containing parabens. Generally, all parabens modified the natural aggregation behavior of HPβCD and γCD as the apparent critical aggregation concentration (cac) values for paraben/CD systems decreased compared to those of pure aqueous CD solutions. The longer the alkyl side chain, the greater was the promotion of aggregates formation (methyl < ethyl < propyl < butyl) and, consequently, more and larger aggregates are formed. These superstructures are responsible for the observed changes in apparent cac and flux values, as well as, for the observed slopes greater than unity for the phase-solubility diagrams.

Keywords Cyclodextrin · Osmolality · Aggregation · Permeation · Drug delivery system

Introduction

Cyclodextrins (CDs) are a group of water soluble macrocyclic oligomers of glucose linked by α-(1,4) glycosidic bonds [1, 2]. They are formed by enzymatic degradation of starch and named according to the number of glucose residues α-cyclodextrin (αCD), β-cyclodextrin (βCD) and γ-cyclodextrin (γCD) consisting respectively of 6, 7 and 8 glucose units [3]. Due to their toroidal truncated cone structure, these molecules have hydrophilic outer surfaces and relatively hydrophobic central cavities [4, 5]. These features make them quite versatile and useful as they are relatively soluble in water and at the same time capable of inclusion complex formation i with a wide range of different lipophilic or partly lipophilic guest molecules [5, 6]. Formation of guest–host inclusion complexes (e.g., drug–CD complexes)

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can improve the apparent physicochemical properties of the guest such as its chemical stability, bioavailability, dissolution rate in aqueous media and aqueous solubility [7–9].

Poorly water-soluble molecules, like parabens (family of alkyl esters of 4-hydroxybenzoic acid), are good candidates as guests for inclusion complex formation with cyclodextrins. Parabens are named according to the alkyl chain methyl (MP), ethyl (EP), propyl (PP) and butyl paraben (BP). The alkyl chain length determines the aqueous solubility of parabens, as well as, their ability to interact with CDs [10–13].

Several research publications describe complexation of parabens with αCD [14, 15], βCD [10, 11, 14–17], γCD [12], HPαCD [10], HPβCD [10, 13, 18–21], SBEβCD [22] and HPγCD [22]. However, only few describe the self-aggregation behavior of these CDs and their complexes in presence of parabens [12, 13].

Currently self-assembly of CDs and their complexes is a hot research topic and has opened a new perspective on CD usage [13, 23, 24]. On one hand, it can be advantageous for pharmaceutical formulators since self-assembly is a simple and versatile method for creation of novel drug delivery systems, but on the other hand self-assembly of CDs and their complexes can be disadvantageous since it may lead to formulations with insufficient stability. Consequently, increased knowledge on how guest compounds influence CD and CD complex aggregation is highly valuable. Methods that stabilize complex aggregates can promote formation of novel drug delivery systems and methods that prevent their formation can be applied to stabilize CD-containing drug formulations.

The aim of this present study is to access changes of the physiochemical properties of paraben/CDs complexes, such as their solubility and aggregation, induced by the presence of parabens using permeation and osmolality methods.

Materials and methods

Materials

αCD, βCD, γCD and 2-hydroxypropyl-β-CD (HPβCD) of degree substitution (DS) 4.2 (MW 1380) were kindly provided by Janssen Pharmaceutica (Beerse, Belgium). 2-hydroxypropyl-α-CD (HPαCD) DS 3.6 (MW 1180) and 2-hydroxylpropyl-γ-CD (HPγCD) DS 4.2 (MW 1540) were purchased from Wacker Chemie (Burghausen, Germany). Methyl- (MP), ethyl- (EP), propyl- (PP), and butyl- (BP) paraben were kindly provided by Janssen Pharmaceutica (Beerse, Belgium). Milli-Q water (Millipore, Billerica, MA) was used to prepare both CD solutions and mobile phases. The solvent used for analysis (acetonitrile) was of HPLC grade and obtained from Sigma-Aldrich (St. Louis, Missouri, USA).

Paraben/CDs samples

The phase-solubility studies were described in part 1 of this two-part series [25]. Here the phase-solubility of the four parabens were studied in aqueous solutions containing the natural αCD, βCD and γCD as well as their hydroxypropylated derivatives. The liquid phases obtained from the phase-solubility studies were used as sample solutions for the osmolality and permeation studies.

Quantitative determination of CD/paraben samples

A reverse-phase ultra high-performance liquid chromatography system (UHPLC) from Dionex Softron GmbH (Germering, Germany) was used for determination of both CDs and parabens concentrations. Ultimate 3000 series consisting 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 (stationary phase) with a matching HPLC Security Guard (Phenomenex, Cheshire, UK) were used. The mobile phase consisted of acetonitrile and water (50:50). The flow rate was set to 1.0 mL/min and temperature of the column to 30 °C. The injection volume was 10 µL. Chromatograms were evaluated using ChromeleonR version 7.2 SR4 (ThermoFisher Scientific, MA, USA). The Corona detector allowed quantification of both CD and the paraben [25].

Osmolality measurements

OSMOMAT 30 (Gonotec GmbH, Germany) freezing point osmometer was used. The osmometer was calibrated at 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. Only 50 µL of solution was required. All samples were measured immediately after filtration of aliquots sampled from the phase-solubility systems (to avoid precipitation of components during storage).

Permeation studies

The effect of parabens on apparent cac values of γCD and HPβCD was determined by the permeation method using unjacketed Franz diffusion cells with diffusion area of 1.77 cm^2 (SES GmbH—Analyse systeme, Germany). Aqueous solutions saturated with a given paraben, obtained from the phase-solubility studies, were used as donor phases (2 mL), while MilliQ autoclaved water (to remove dissolved

air) was used as the receptor phase (12 mL). The donor and receptor compartments were separated by 3.5–5 kDa molecular-weight-cutoff (MWCO) semi-permeable cellulose ester membrane (Biotech CE, Spectrum Europe, Breda, NL). The experiments were carried out at room temperature (i.e. 22–23 °C). The receptor phase was stirred continuously with magnetic stirrer operated at 300 rpm whereas the donor phase was unstirred. Samples (150 µL) were collected from the receptor phase every 15 min post lag time (1 h for γCD and 0.25 h for HPβCD): from 60 to 120 min for γCD and from 15 to 75 min for HPβCD. Samples were immediately replaced with equal volume of fresh MilliQ water. CD and parabens were quantified using UHPLC. The calculation of steady state flux (J) of the paraben/CD was obtained from the slope (d_q/d_t) of the linear regression relationship between time (t) and the amount of CD and paraben in receptor chamber (Eq. 1):

$$J = \frac{dq/dt}{A} = P_{app} \cdot C_d \quad (1)$$

where A is the diffusion area (1.77 cm^2) and C_d is the total paraben/CD concentration in the donor phase. In order to assure that experiments were done under sink conditions [26, 27], volume changes and final concentration of components in donor phases were determined.

Apparent cac for 3.5–5 kDa (i.e. the concentration at which the aggregates size becomes larger than the pore size, leading to a deviation of ideal flux from CD through the membrane) was calculated by drawing tangent lines in the flux graphics.

Results and discussion

In first part of this work [25] complexation between CDs and parabens in solution and solid phase was demonstrated by diverse analytical methods: phase solubility studies, differential scanning calorimetry, Fourier transform infrared spectroscopy and X-ray powder diffraction. Factors such as the paraben alkyl side chain and the CD cavity size were shown to have impact on the solubilization and complexation efficiency of these systems. In this second part, osmometry and permeation studies were performed to evaluate if and how parabens affected the flux and apparent critical aggregation concentration (cac) of the studied CDs up on inclusion complex formation, as well as, how the CDs complexes influenced permeation of the studied parabens.

Osmolality measurements

As this is a method based on colligative properties and osmolality is directly related to the number of dissolved particles in the solution, osmometry can be used as alternative method analyze and determine phase-solubility diagrams. Previously we have shown that osmolality measurements can be applied to detect CD aggregates [28, 29]. Therefore, this analytical method was applied to detect aggregates of paraben/CD inclusion complex solutions. Osmolality of CD stock solutions and CD solutions saturated with parabens were plotted against initial CD concentrations.

Native cyclodextrins

Generally, samples containing paraben complexes of the native αCD, βCD and γCD displayed negative osmolality deviations in comparison to pure aqueous αCD and γCD solutions (Fig. 1a, b). These observations point to formation of poorly soluble paraben/CD complexes at certain CD

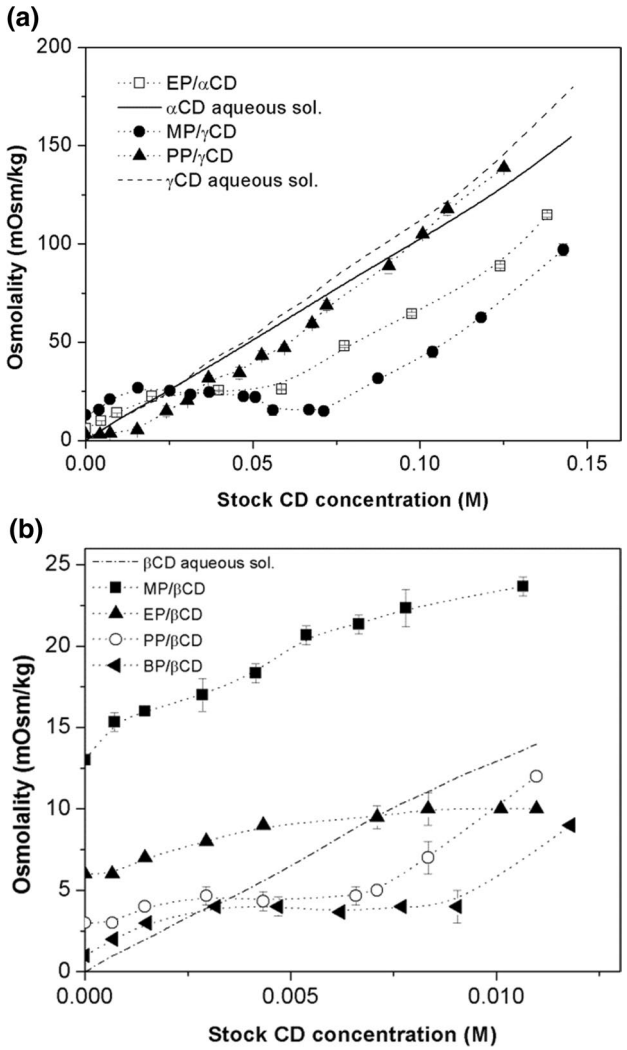

Fig. 1 **a** Changes of total osmolality of some of the prepared αCD/paraben and γCD/paraben systems during phase-solubility experiments (25 °C). **b** Changes of total osmolality for the βCD/paraben systems during phase-solubility experiments (25 °C). Symbols represent mean ± SD (n = 3)

concentrations. Indeed, all parabens added to the aqueous αCD and γCD solutions caused depression of osmolality (decrease of particle number due to precipitation of complex) (Fig. 1a), which is in accordance with phase solubility studies that were determined to be of B-type.

Figure 1a shows how the osmolality changes for three of the studied paraben/native CD systems. We observe two examples of a B_S-type profile (EP/αCD and MP/γCD) that can be divided into three characteristic regions. At low CD concentrations, osmolality of the systems is higher than that of the CD stock solution due to dissolved paraben and, consequently, contribution of parabens to total osmolality. Such effect continues until a certain concentration of CD (crossing point with the CD curve) where formation of poorly soluble paraben/CD complexes starts (plateau region) and negligible change of overall osmolality can be observed. As soon as no solid paraben is available in the system, the osmolality increases again due to increased concentration of free CD in the aqueous complexation medium. The PP/γCD complex system displays B_i-type phase-solubility diagram [25], which presents a quite similar behavior to the B_S-type diagram. However, the plateau region starts from the beginning since the formed PP/γCD complexes are so insoluble that precipitation process takes place even at very low γCD concentration.

In the case of βCD (Fig. 1b), phase-solubility diagrams are of A_L-type for MP/EP and B_S-type for PP/BP [25]. Due to the low aqueous solubility of βCD, presence of parabens in the complexation medium will increase the osmolality of the dilute βCD solutions. For MP and EP, a slightly constant increase of osmolality is observed through the βCD concentration range, though it tends to plateau at higher CD concentrations (probably due to slight precipitation of βCD). However, PP and BP display B_S-type profiles and show osmolality changes similar to those described above.

Contrarily to what happened in case of the CD single systems, no conclusions can be obtained from the osmolality measurements regarding the possible effect of complexes on the aggregation process of native CDs. Influence of parabens on the total osmolality of the systems and precipitation of insoluble complexes cause uncertainties regarding correlation between osmolality deviations and formation of aggregates. However, determination of osmolality changes can be applied to determine phase-solubility profiles of the tested systems.

Hydroxypropylated cyclodextrins

As seen in Fig. 2, the osmolality of the aqueous HPαCD, HPβCD and HPγCD solutions does not change significantly upon saturation with parabens. In other words, the total concentration of osmotically active particles remains the same or slightly higher in comparison with the initial state. This observation excludes formation of poorly soluble paraben/CD complexes and, consequently, their precipitation. Thus,

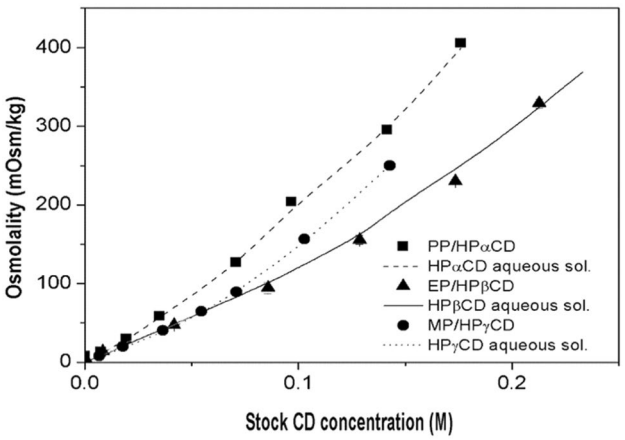

Fig. 2 Illustration of total osmolality changes for some of the prepared paraben/HP-CD systems during phase-solubility experiments (25 °C). Symbols represent mean ± SD (n = 3)

the osmolality measurements confirm our results from the phase-solubility studies showing that the hydroxypropylated CDs displayed A_L-type profiles [25]. In this case, the osmolality measurements cannot provide any useful information about aggregation of formed paraben/HP-CD complexes.

Solubility of native and HP derivatives in the paraben media

In order to find a plausible explanation for osmolality depression, added (initial) and actual (final) CDs concentrations during phase-solubility experiments were plotted against each other (Fig. 3). Bisectors of those plots (i.e. the solid lines) correspond to the theoretical situation where there are no changes in CD concentrations. The studied systems corresponding to A_L-type profiles, MP/βCD and EP/βCD (Fig. 3b) and system containing the hydroxypropylated derivatives (Fig. 4), did not show any difference between initial CD concentration and the concentration of dissolved CD after addition of parabens. The highly soluble nature of the formed complex is responsible for the absence of CD precipitation reason why solubility of CDs did not alter. However, we believe that the observed A-type phase-solubility diagrams for βCD with MP and EP are only due to the very low water solubility of βCD.

In all other cases, systems containing the natural CDs displayed CD precipitation (Fig. 3). This phenomenon occurs when CD concentration of reaches the solubility limit of the formed complex in the paraben saturated solution that is when the plateau region is attained. Additional amount of CD results in precipitation of formed complex until no solid paraben is available in the complexation medium. Thereafter the concentration of dissolved CD in the aqueous medium will increase until it has been saturated with CD.

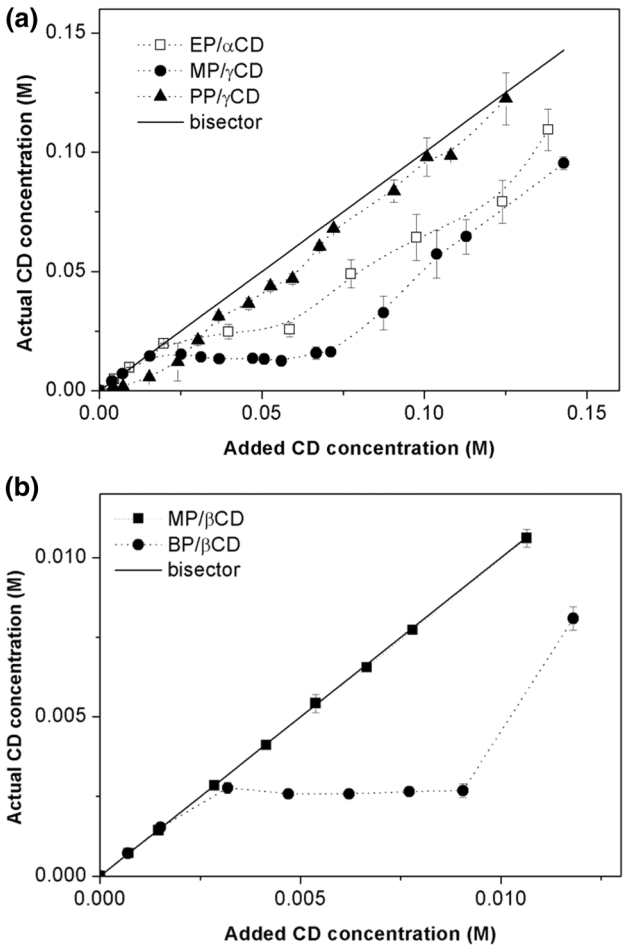

Fig. 3 Illustration of αCD and γCD (**a**) and βCD (**b**) solubility in some of the prepared paraben/CD systems. Symbols represent mean ± SD (n = 3)

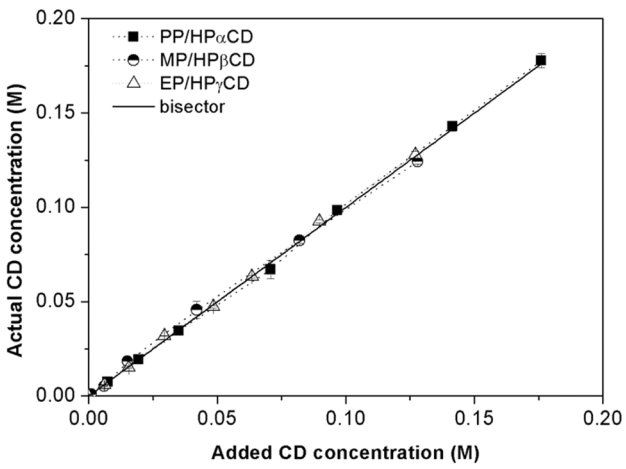

Fig. 4 Illustration of HP-CDs solubility in some representative paraben/HP-CD systems. Symbols represent mean ± SD (n = 3)

This description is typical for B_S-type profile: all parabens/αCD, PP/βCD, BP/βCD, MP/γCD and EP/γCD [25]. In the case of the B_i-type profiles (PP/γCD and BP/γCD) the CD molecules will form insoluble paraben complexes from the beginning of the profile. Only when all solid paraben has been removed from the media, the concentration of dissolved CD starts to increase (Fig. 3).

Permeation studies

For the permeation studies, two sets of parabens complexes were selected: γCD and HPβCD complexes as representatives of CDs forming B_S-type and A_L-type profiles.

Hydroxypropylated βCD

In order to evaluate the effect of parabens on the HPβCD aggregation behavior, permeation studies were performed using the liquid phases from the phase-solubility experiments. The flux of HPβCD from the paraben/HPβCD systems was plotted versus HPβCD concentration (Fig. 5) and the apparent cac values for 3.5–5 kDa membrane were determined (Table 1). As previously described, the apparent cac value determined using the permeation method does not represent the CD concentration where aggregates start to be formed, but rather the CD concentration at which their size becomes greater than the pore size of the membrane used.

These experiments were carried out under sink conditions [26] since changes in donor phases volume were insignificant (always < 0.1 mL) and HPβCD concentrations in the donor phases at the end of the experiment was always greater than 90% of initial concentration.

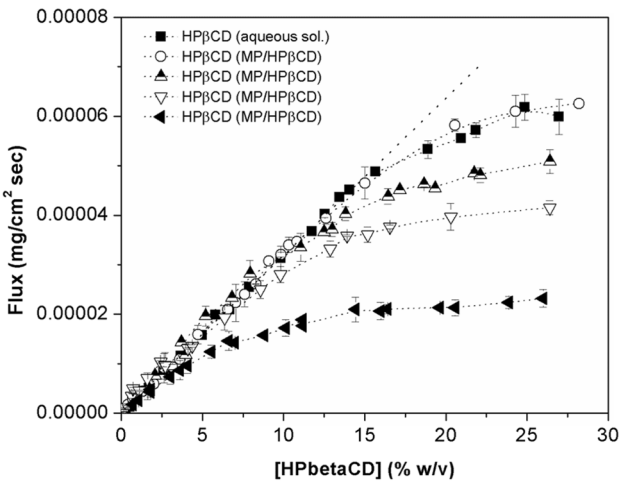

Fig. 5 Flux profiles of HPβCD from pure aqueous HPβCD solution and from different paraben/HPβCD systems (liquid phases). All permeation experiments were performed at room temperature using 3.5–5 kDa MWCO semipermeable membranes. Symbols represent mean ± SD (n = 3)

Table 1 Calculated apparent cac values for HPβCD and γCD in pure water and in presence of tested parabens for membrane MWCO 3.5–5 kDa pore size

Paraben	HPβCD cac %(w/v)	γCD cac %(w/v)
None*	11.8	4.2
MP	11.7	1.4
EP	9.5	0.9
PP	8.6	4.6
BP	5.5	4.5

*Apparent cac values determined for pure aqueous CD solutions

Previously we determined the HPβCD flux profile from pure aqueous HPβCD solutions as represented in Fig. 5 [29]. Regarding the membrane used in the present study (MWCO 3.5–5 kDa) we observed a linear increase of HPβCD flux with HPβCD concentration until 11.8% w/v. At lower HPβCD concentrations the size of the HPβCD aggregates was smaller than the pore size of the membrane and, thus, HPβCD did permeated freely through it. After the apparent cac had been reached, a negative deviation from linearity was observed meaning that size of aggregates started to be too large for them to pass freely through the membrane in to the receptor phase.

When comparing the flux curve of an aqueous HPβCD solution and of HPβCD from an aqueous MP/HPβCD system, no significant difference between them is observed (Fig. 5). The presence of MP in the system does not seem to affect the self-assembly of HPβCD as a relative similar values of apparent cac (11.8 vs. 11.7%) was registered (Table 1). The formation of MP/HPβCD complex had no or limited influence on size of the aggregates as observed by the overlapping of flux curves for the two systems. Stappaerts et al. also reported a limited effect of the short alkyl side chain of MP on the HPβCD aggregation [13].

Contrarily to MP, the remaining three parabens all affected the HPβCD aggregate formation. Parabens with the longer alkyl side chains affect the HPβCD aggregation and the longer it is the lower is the apparent cac value (Table 1). Thus, size of aggregates gradually increased with increasing chain length (MP < EP < PP < BP). The flux values also decreased with increasing paraben alkyl chain length as it can be observed in Fig. 5 (curve for HPβCD from MP/HPβCD displayed higher flux values than EP/HPβCD > PP/HPβCD > BP/HPβCD).

Native γCD

The permeation method was also used to investigate if parabens affect the aggregation process of γCD. As in previous section, γCD flux for the studied paraben/γCD systems

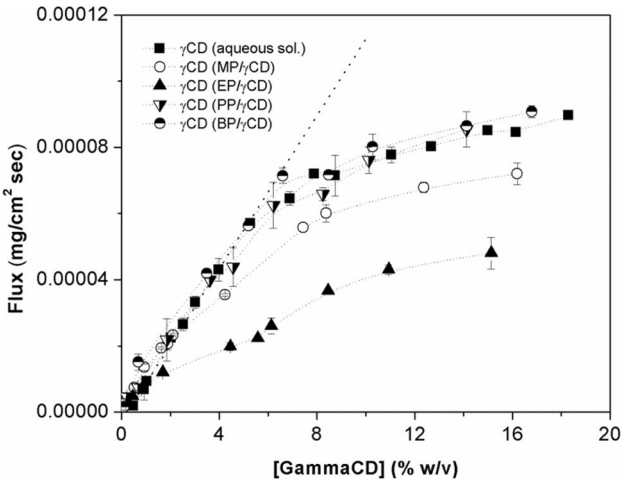

Fig. 6 Flux profiles of γCD from pure aqueous γCD solution and from different paraben/γCD systems (liquid phases). All permeation experiments were performed at room temperature using 3.5–5 kDa MWCO semipermeable membranes. Symbols represent mean ± SD (n = 3)

was calculated and then plotted versus γCD concentration (Fig. 6). Apparent cac values for each system were also determined (Table 1). These experiments were also carried out under sink conditions [26, 27] as changes in donor phases volume were insignificant (always < 0.1 mL) and γCD concentration in donor phase at the end of each run was always > 90% of initial concentration.

First, we studied and plotted the flux profile from aqueous γCD solutions (black squares). The 3.5–5 kDa MWCO membrane was selected in the present study. The tangent line in Fig. 6 shows that a linear increase of flux with concentration is observed approximately until 4.2% w/v (apparent cac value of γCD in pure water). Below 4.2% γCD the aggregates were small enough to permeate freely through the membrane. Above 4.2% γCD, a negative deviation from linearity was observed due to formation of γCD aggregates that were unable to permeate the membrane.

Previously it was shown that γCD formed B_s-type phase solubility diagrams with MP and EP, while PP and BP formed B_i-type phase solubility diagrams [25]. Consequently, the PP/γCD and BP/γCD systems displayed different γCD flux curves. Table 1 shows that apparent cac values for aqueous γCD solutions containing MP/γCD and EP/γCD inclusion complexes changed significantly (to 1.4 and 0.9% w/v, respectively) when compared to the apparent cac value for solution containing only γCD (4.2% w/v). These two parabens had a clear influence on formation of γCD aggregates. In their presence, γCD started to form larger aggregates at lower γCD concentrations. These larger structures are responsible for the fact that flux dropped and gave negative deviation from linearity at low γCD concentrations.

Results suggest that MP and EP promoted self-aggregation process of γCD (Fig. 6).

The PP/γCD and BP/γCD systems showed some similarities to the permeability behavior of pure aqueous γCD solutions. Contrarily to the two previously described systems, the flux curves of γCD from PP/γCD and BP/γCD systems almost overlapped the one obtained from the pure γCD solutions showing just small difference in terms of apparent cac value. The values are slightly higher (i.e. 4.6 and 4.5%w/v for PP and BP/γCD, respectively) than for pure aqueous γCD solution. However, due to standard deviations we can affirm that these apparent cac values are not different. With these results, we could conclude that these longer alkyl chain parabens do not affect the γCD aggregation process. However, due to the type of phase-solubility profile determined for these systems (i.e. B_i-type), conclusions need to be taken with caution. Knowing that parabens were added to γCD solution just in the amount needed to assure the formation of solid phase in equilibrium stage (and not in significant excess), and knowing that saturation concentration of these inclusion complexes (PP and BP/γCD) is easily achievable at low γCD concentrations, the amount of PP and BP inclusion complex in liquid phase will be reduced (even below limit of quantification). This analytical limitation made the determination of possible influence of these complexes on the self-assembly of γCD difficult, as the liquid phase of these systems mainly contained free γCD.

Figure 7, which shows the ratio of γCD in liquid phase present before addition of the paraben (added γCD concentration) and at the end of phase solubility experiment (actual γCD concentration after equilibrium), proves exactly this. It can be seen at end of phase solubility experiment (equilibration time) that most frequently 80–90% of added γCD concentration is still present in the system. This can explain why the flux profiles and the apparent cac values for γCD in presence of these two parabens are quite similar to the one determined for pure aqueous γCD solutions.

All liquid phases from phase-solubility studies (i.e. the donor solutions for permeation studies) were subjected to centrifugation in order to obtain better separation of the solid fraction from the solution, before the filtration step. We believe that aggregates are present in both PP/γCD and BP/γCD systems but due to large size and low percentage, they might have precipitated during the centrifugation step. It is therefore difficult to conclude something about the influence of PP and BP on aggregation process of γCD.

Influence of HPβCD and γCD on permeation behavior of parabens

Above the effect of parabens on the permeation and aggregation profiles of HPβCD and γCD was discussed. Since both the parabens and CDs were simultaneously analyzed we were able to study how the CDs and their complexes influenced the permeation profile of the parabens.

HPβCD and γCD, due to formation of soluble paraben/CD complexes, were both capable of increasing the paraben flux through the semi-permeable membrane in comparison to the paraben flux from pure aqueous paraben solutions. Figure 8 shows the paraben flux profiles versus the paraben concentration for HPβCD complexes (Fig. 8a) and the MP/γCD inclusion complex (Fig. 8b). For the latter, we were only able to study one system (MP/γCD) as concentration of remaining complexes (EP/γCD, PP/γCD and BP/γCD) in liquid phase was close to or below the detection limit for parabens in the receptor phase.

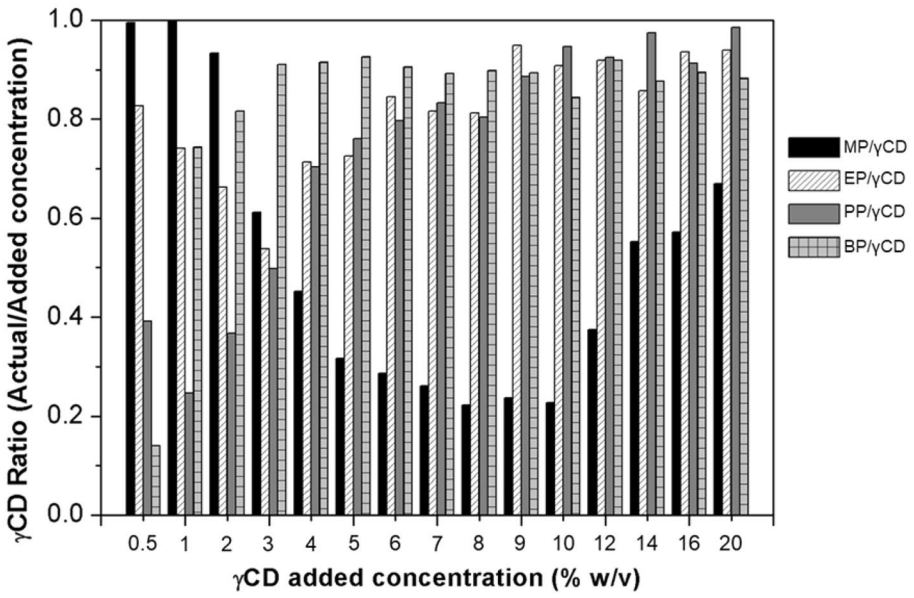

Fig. 7 Variation of γCD ratio (actual/added concentration) with added (theoretical) γCD concentration for different paraben/γCD systems

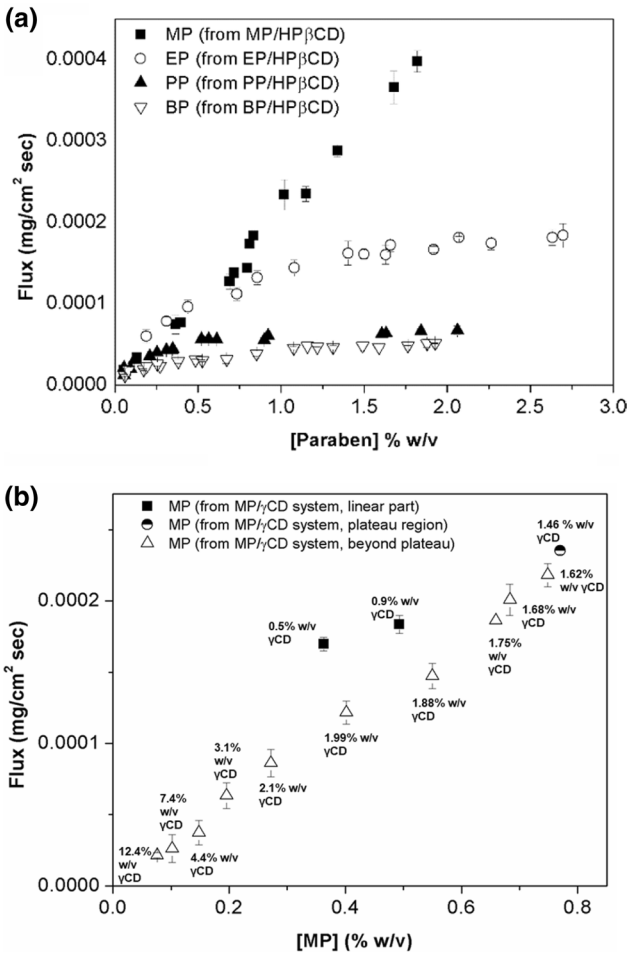

Fig. 8 Permeability profile of parabens in paraben/HPβCD systems (**a**) and of MP in MP/γCD (**b**) through 3.5–5 kDa MWCO semi-permeable membrane (room temperature). Symbols represent mean ± SD (n = 3)

The different parabens displayed different flux values from the aqueous HPβCD solutions. Figure 8a shows that HPβCD, through complex formation, linearly increased MP flux from the donor to the receptor chamber with increasing MP concentration (following Fick's first law).

Table 1 shows a decrease of apparent cac values for HPβCD promoted by the presence of EP, PP and BP. These three parabens promote formation of larger size HPβCD aggregates making them too large to penetrate freely through the 3.5–5 kDa pore. The larger the alkyl chain the larger are the aggregates formed and the more the paraben flux will drop. Formation of complex aggregates in the aqueous HPβCD solutions containing EP, PP and BP influenced the parabens' flux through the semi-permeable membrane.

The phase-solubility profile (B_S-type) might be the reason why MP flux diagram of the MP/γCD system had a peculiar shape. The two first illustrated points ($0.36\%/1.7\times10^{-4}$ and $0.49\%/1.8\times10^{-4}$) (Fig. 8b) correspond to the linear part of the phase-solubility profile. At this stage, MP water solubility increased linearly with increasing γCD concentration (formation of MP/γCD inclusion complexes) as well as the MP flux through the membrane. Furthest to right on the flux graph (Fig. 8b, $0.77\%/2.4\times10^{-4}$) shows the point displaying the highest MP flux which in fact corresponds also to the highest apparent solubility of MP/γCD complex. Then a descending part can be defined on the graphic due to precipitation of MP/γCD complexes (liquid phases here analyzed were already from plateau region and beyond). However, during this decrease of MP flux the γCD concentration is constant in the plateau region after which it slowly starts to increase again. If γCD would not have any influence on the permeation of this paraben (other than facilitate it due to increase of apparent solubility), we would see an overlapping of flux points. This is because we have samples with exactly same amount of MP. However, those samples containing similar amounts of MP had also different γCD concentrations, which can explain the graph's peculiar shape. Larger aggregates were formed with increasing γCD concentration. These assembled into super structures promoting eventually the precipitation of inclusion complexes and, consequently, the decrease of MP in the donor phase. The presence of these large aggregates in donor phase also hampered the passage of components from donor to receptor phase leading to the MP flux decrease that is registered in the graphic.

Analysis of Fig. 8b, might lead us to think that we can get develop systems with complexes with the same concentration of MP at different concentration of γCD but with different permeation profile. This discovery can be an extremely useful tool for pharmaceutical formulators working with ocular or dermal delivery. Just by choosing different concentration of γCD they will be able to produce different drug delivery systems. It will be possible to produce delivery systems where components will permeate faster (aggregates present) or sustained-permeation delivery systems due to γCD aggregates.

Conclusions

Overall osmolality results provided valuable information and confirmed analysis of the phase solubility profiles for all the paraben/CD systems studied. No changes in total osmolality were detected for systems displaying A_L-type phase-solubility diagrams, while for systems displaying B-type diagrams changes were registered mainly due to precipitation of the complex. When looking at the paraben contribution to the changes in CD solubility, A-type systems (MP/βCD, EP/βCD and HP-CD derivatives) did not display any difference between initial and final CD concentration. On the other hand, parabens did affect the solubility of the native CDs leading to precipitation of solid complexes. The

osmometric method could not be applied to study the effect of complexes on the aggregation process of native CDs, however, it was shown to be an easy and feasible method to corroborate or determine phase-solubility profile of the tested system.

Of all the methods tested, the permeation studies were the best for evaluation of self-assembly of CDs. It was possible to conclude that paraben alkyl chain had an important influence on HPβCD (except with MP) and γCD tendency to self-aggregate, the longer this side chain, the higher is the promotion of aggregate formation which leads to a gradual increase of CD particle size. This increase of aggregates size is the main reason for the changes of apparent cac and flux values (MP < EP < PP < BP) registered during this study.

The effects of PP and BP on γCD aggregation could not be studied by the permeation method. Even though the apparent cac values for the 3.5–5 kDa membrane and flux profiles were similar to those obtained for pure aqueous γCD solutions, we believe that these parabens are also capable of promoting formation of aggregates. However, these aggregates might have been disassembled during sample preparation by centrifugation due to their extremely large size and low concentration.

Not only did the parabens affect the physiochemical properties of the CD complexes but also the CDs did affect the physiochemical properties of the parabens. The results show that HPβCD and γCD complex aggregates increased the parabens' solubility and influenced their flux through semi-permeable membranes but in a different way. MP/HPβCD complex aggregates were always small enough to facilitate and increase the flux of MP throughout the CD concentration. Instead, due to their size (at least larger than 3.5–5 kDa), the complex aggregates formed of all the remaining studied liquid phases were responsible for the visible delay of the respective parabens' flux.

Overall, we can conclude that guests with different physicochemical properties (e.g. different lipophilicity due to differences in alkyl chain length) can influence the apparent solubility and tendency of paraben/CDs complexes to aggregate.

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