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ORIGINAL ARTICLE

Exploring the interactions between buffers and cyclodextrin complexes—formation of regular inclusion or atypical non-inclusion complexes

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Abstract
The aim of this study was for the first time to determine the effect of 11 buffers on a γ-cyclodextrin complex, and use these and previous reported data to systematically explore the effect of buffers on different cyclodextrin complexes, considering differences in cavity size and exterior between the cyclodextrins. The effect of 11 buffers on the binding between γ-cyclodextrin and the bile salt taurochenodeoxycholate was determined using isothermal titration calorimetry, and the stability constant of the complex ranged from 6.1×10^4 to 9.0×10^4 M^{-1}, depending on the buffer species. Three buffers (citric, maleic and 2-morpholinoethane-sulfonic acid) decreased the stability constant of the complex compared to the stability in water, though to a degree that has limited practical relevance. As for other cyclodextrin complexes, the stability constant depended on the buffer species present in solution. The analysis showed that the size of the cyclodextrin cavity, rather than the exterior, was paramount for the effect of carboxylic acid buffers, suggesting formation of regular inclusion complexes between carboxylic acid buffers and cyclodextrins.

Keywords Binding constant · Host–guest chemistry · Driving forces · Equilibrium · Complexation

Introduction

Cyclodextrins (CDs) are macrocyclic molecules produced by starch, and they consist of glucose units linked through 1,4-α-glycosidic bonds [1, 2]. The chemical structure yields a cone-like structure with a hydrophobic cavity and a hydrophilic exterior, which is central for all interest in these molecules [3]. CDs are able to form inclusion complexes with small, hydrophobic guest molecules by inclusion in their cavity, shielding the hydrophobic molecules from the external environment. Naturally produced CDs include α-, β- and γ-CD, which consist of 6, 7 and 8 glucopyranose units, respectively [4]. Consequently, the natural CDs have varying cavity sizes of 4.7–5.3, 6.0–6.5, and 7.5–8.3 Å in diameter for α-, β- and γ-CD, respectively [5]. Their ability to form inclusion complexes is strongly linked to the fit between CD cavity and guest molecule [6, 7]. It is often said that α-CD complexes well with aliphatic chains, β-CD with aromatic molecules and γ-CD with larger guest molecules [2, 8], though one guest molecule may be able to form complexes with more than one natural CD, since guest molecules are often only partly included in the CD cavity.

Until now, β-CD remains by far the most investigated CD, though, recently γ-CD is gaining more attention due to its low toxicity and its larger cavity size [9]. In addition to the natural CDs, many CD derivatives have been synthesized by substituting hydroxyl groups of the CD with hydrophilic moieties [6]. Common derivatives include hydroxypropyl- and sulfobutyl ether-derivatives [10]. Both of these derivatives have high aqueous solubility and low toxicity.

Previous studies have demonstrated that buffers may affect the apparent stability constant for β-CD, HP-β-CD and SBE-β-CD complexes [11–17], however, little is known about the effect of buffers on γ-CD complexes. This is unsurprising, as β-CD has been more thoroughly

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investigated. The use of γ-CD is increasing [9], and it is relevant to investigate potential interactions between γ-CD and buffers used in CD research. We have previously reported that the effect of some buffers on natural and modified β-CD complexes is linked to a competitive mechanism of the buffers [16, 17], but it remains unclear whether buffers interact with the CD cavity through formation of regular inclusion complexes or with the exterior of CD through atypical non-inclusion complexes, also referred to as association complexes. It has been suggested that inclusion formation between CDs and hydrophilic buffers is possible [18], but it is difficult to prove without doubt. Previous studies reported interactions between carboxylic acid buffers and β-CD [18–21], and two studies have used ROESY NMR to demonstrate interaction between some buffers, i.e. citric, succinic, maleic, tartaric and fumaric acid, and protons in the β-CD cavity [11, 16], supporting formation of regular inclusion complexes. However, the interactions shown by NMR are very weak, and the results are ambiguous.

The present study aims to investigate the effect of 11 buffers on the interaction between γ-CD and the bile salt taurochenodeoxycholate (TCDC). The bile salt was used to illustrate proof of concept, since TCDC forms strong inclusion complexes with β- and γ-CDs [2, 22], making it a good model guest. The interaction between TCDC and α-CD is very weak [23], hence, it is not relevant to study the effect of buffers on this system. Additionally, this study aimed to explore the hypothesis regarding buffers interaction with CD complexes. Do buffers interact with CDs through regular formation of inclusion complexes? Or do they form non-inclusion complexes through hydrogen bonds on the exterior of the CD? If unconventional association complexes are formed between buffer and CD, the same magnitude of interactions should be expected for the various buffers on both β- and γ-CD complexes due to the similarity of the exterior of the two CDs. On the other hand, if inclusion complexes are formed, the different size of the β- and γ-CD cavity will influence the interactions.

Materials and methods

Reagents

Taurochenodeoxycholate sodium salt (CAS: 6009-98-9, $M_w = 521.7$ g mol^{-1}) and γ-CD (CAS: 17465-86-0, Mw = 1297.12 g mol^{-1}) were purchased from Sigma-Aldrich and used as received.

Preparation of buffers

Buffers were prepared in stock solutions of 100 mM in milliQ water, with small adjustments of either sodium hydroxide or hydrochlorid acid to achieve the desired pH of each buffer solution. The pH value of a solution may significantly affect the binding constant of CD complexes, if the guest or CD is ionizable [24, 25]. In this study, the ionization state of CD and TCDC was not influenced. The pH value of the buffers differed, and the effect of pH was minimized by, for each buffer, selecting the pH, which favored the presence of the neutral buffer species, since the potential interactions between CD and buffers was expected to be greatest in this situation. However, the pH was never lower than 2.5, due to compatibility with the equipment. The buffers used and the pH of the solutions can be seen in Table 1.

Determination of apparent stability constants

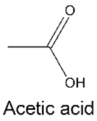
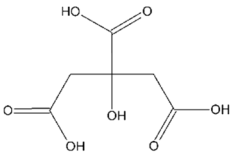
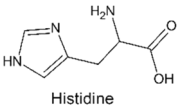
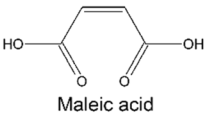
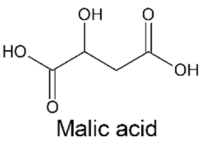
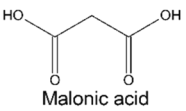
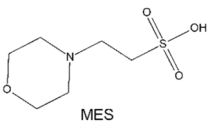
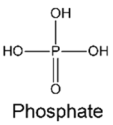
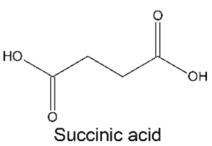
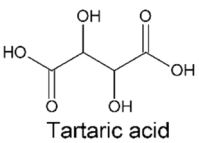
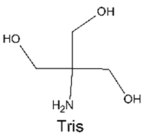
Isothermal titration calorimetry (ITC) was used to determine the apparent stability constants (K) as well as the enthalpy (ΔH) and entropy (ΔS) of reactions for γ-CD and TCDC in the buffers. Measurements were performed using a MicroCal VP-ITC MicroCalorimeter (Malvern Panalytical, Worcestershire, UK), and all experiments were conducted at 25 °C. Solutions of 0.25 mM TCDC in milliQ water or buffer was prepared. The concentration of TCDC was below the critical micelle concentration (cmc) [26], and thus aggregation of TCDC was negligible. Correspondingly, solutions of 2.5 mM γ-CD was prepared. TCDC was loaded into the sample cell (1.4257 mL), and the CD was loaded into the syringe (250 µL). The solution in the sample cell was continuously stirred with a speed of 310 rpm. The titrations started with one addition of 2 µL, which was the neglected in the analysis, and continued with 30 injections of 10 µL each. The injection time was 20 s, and 200 s were allowed between injections. Blank titrations of CD into buffer were used to correct for heat of dilution.

Results and discussion

ITC data

A total of 11 buffers was investigated with respect to their effect on the complexation between γ-CD and TCDC. The stability constant (K) and the change in enthalpy (ΔH) of the reaction for the complex were determined by ITC at 25 °C, and a representative example of the resulting enthalpogram is shown in Fig. 1. The experimental data was fitted to a one-set-of-sites model, which yielded thermodynamic quantities

Table 1 List of main buffer species, structure, pK_a value, and pH of solution used in this study

Buffer species	Structure	pK_a	pH of sol
Acetic acid	 Acetic acid	4.76	3.50
Citric acid	 Citric acid	3.13, 4.76, 6.39	2.53
L-Histidine	 Histidine	1.70, 6.04, 9.09	7.38
Maleic acid	 Maleic acid	1.90, 6.07	2.78
Malic acid	 Malic acid	3.51, 5.03	3.10
Malonic acid	 Malonic acid	2.83, 5.69	2.52
2-Morpholinoethane-sulfonic acid (MES)	 MES	6.15	4.98
Phosphate	 Phosphate	2.16, 7.21, 12.32	5.98
Succinic acid	 Succinic acid	4.21, 5.64	2.70
Tartaric acid	 Tartaric acid	2.89, 4.40	3.76
Tris(hydroxymethyl)-aminomethane (Tris)	 Tris	8.07	8.66

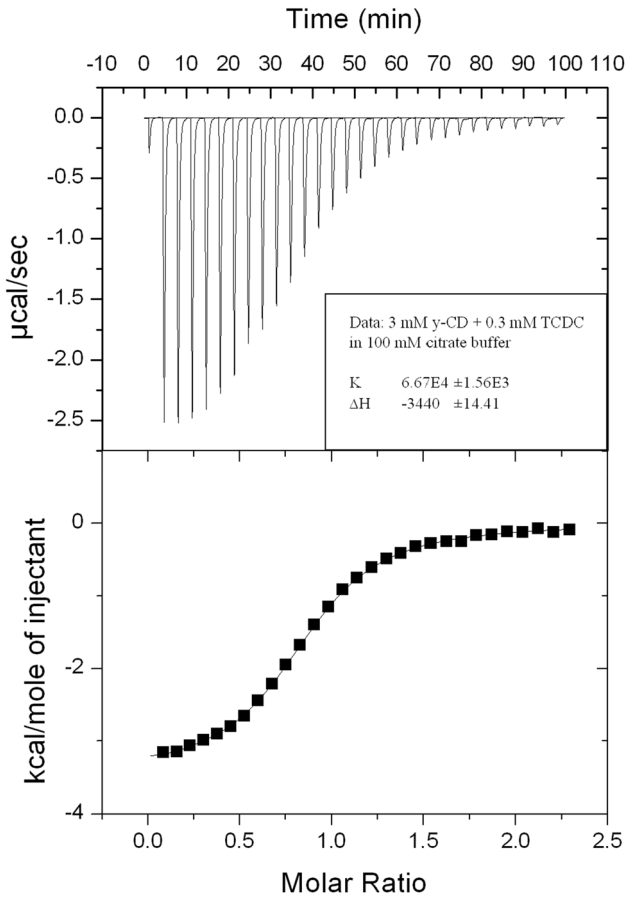


Fig. 1 Representative ITC enthalpogram of 2.5 mM γ-CD titrated into 0.25 mM TCDC in 100 mM citric acid buffer (pH 2.53) at 25 °C. The top part of the figure showed the titration as a function of time, and the bottom part showed the heat of the reaction as a function of the molar ratio fitted to a one-set-of-sites model

with low standard errors (see SI, Table S1). The fit showed excellent agreement with the data points, with a stoichiometry close to 1, indicating a 1:1 reaction between γ-CD and TCDC, supported by findings in a previous study [2]. The average stability constant in water, based on two individual experiments, was 8.0×10^4 M^{-1}, which is similar to a previous reported value of 8.4×10^4 M^{-1} in 50 mM phosphate buffer (pH 7) [2].

The effect of buffers on the apparent stability constant for γ-CD:TCDC

The apparent stability constant was determined in water and each of the 11 buffers listed in Table 1. According to Fig. 2, three buffers appeared to decrease the apparent stability constant of the complex when compared to the stability constant obtained in water, i.e. MES, citric acid, and maleic acid. The three buffers decreased the apparent stability constant by > 15%, with maleic acid showing the greatest effect.

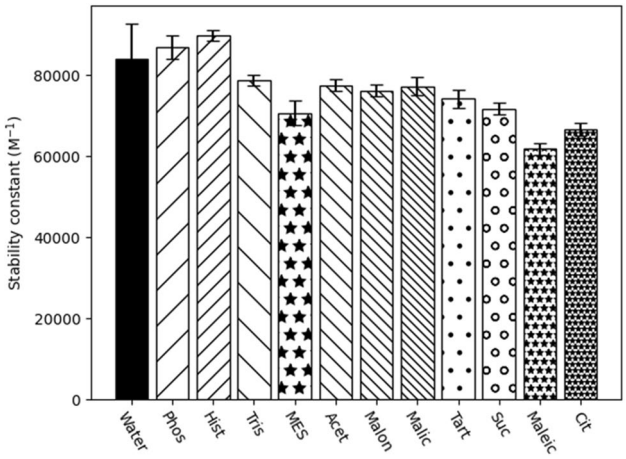


Fig. 2 Apparent stability constants determined by ITC for γ-CD:TCDC complex in water or 100 mM buffer

Though the effect on the apparent stability constant appeared buffer specific (Fig. 2), the decrease in the apparent stability constant was modest at best and with limited practical relevance for the model system of γ-CD:TCDC.

The effects of the buffers were further investigated by studying the enthalpic and entropic contributions to the change in free energy. The results showed that maleic acid was the only buffer which significantly affected the enthalpic and entropic contributions for the complex formation (Fig. 3). The apparent change in Gibb's free energy (ΔG) were similar for all buffers, but for maleic acid, the apparent changes in enthalpy (ΔH) and entropy (ΔS) were higher compared to complex formation in water and the other buffers. Since maleic acid interfered most with the investigated complex, this may indicate that the decrease in the stability constant seen in presence of maleic acid was indeed caused by the buffer. However, at the same time, it is not possible to exclude effect of other buffers, though their effect did not alter the enthalpic and entropic contributions for the complex formation.

Though the effects of the buffers were modest, four buffers were chosen for further investigations, i.e. maleic, citric, succinic, and tartaric acid. These buffers were chosen as two of them (maleic and citric acid) showed moderate decrease in the apparent binding, whereas the other two (tartaric and succinic acid) did not show significant changes to the apparent stability constant. The concentration-dependent effect of the four selected buffers were investigated (Fig. 4). As mentioned previously, the decreases in the stability constant of the γ-CD:TCDC complex in presence of the buffers were modest, however, the data presented in Fig. 4 suggested a concentration-dependent effect. In previous studies, a concentration-dependent decrease in the stability of a CD complexes in presence of other ions was linked to a competitive mechanism [16, 17, 27].

Though the effects of the buffers were modest, the concentration-dependent effects were further investigated. Through nonlinear curve fitting, the ITC data was compared to a theoretical expression for competitive binding. In a previous publication by Holm et al. (2014), competitive binding of an ion was described in terms of a theoretical expression relating the apparent stability constant (K_{app}) and the apparent change in enthalpy (ΔH_{app}) to the stability constants between CD and ion.

$$K_{app} = \frac{K_{CD:Guest}}{1 + C_{Buffer}K_{CD:Buffer}} \quad (1)$$

$$\Delta H_{app} = \Delta H_{CD:Guest} - \frac{K_{CD:Buffer}C_{Buffer}}{1 + K_{CD:Buffer}C_{Buffer}}\Delta H_{CD:Buffer} \quad (2)$$

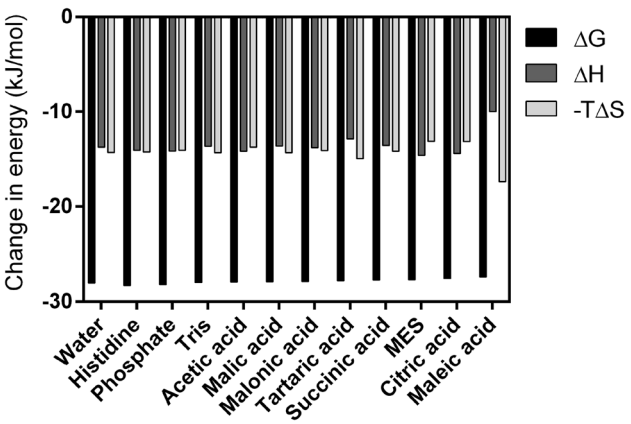


Fig. 3 Apparent enthalpy-entropy compensation determined by ITC for γ-CD:TCDC complex in water or 100 mM buffer

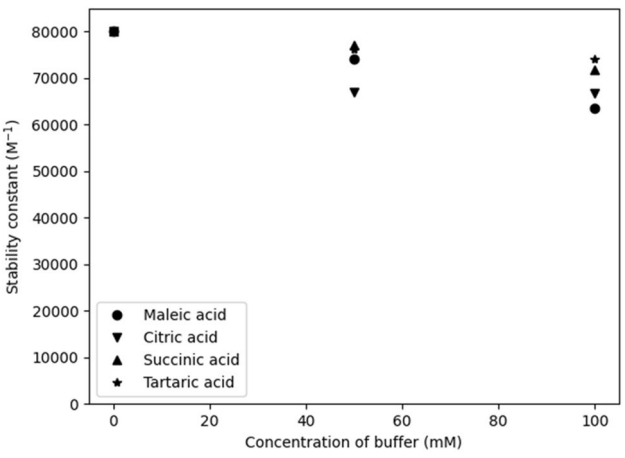


Fig. 4 Stability constant (M^{-1}) as a function of buffer concentration for γ-CD:TCDC in the four buffers; Maleic, citric, succinic and tartaric acid

The stability constant between CD and guest ($K_{CD:Guest}$) was taken as the stability constant for the complex γ-CD in water, and the concentrations of the buffer (C_{Buffer}) were as shown in Fig. 4. The data from the ITC were used as the apparent stability constant and change in enthalpy, and through fitting of the data (Fig. 5), the stability constant between CD and maleic acid ($K_{CD:Buffer}$) was indicated to be an order of 2 M^{-1}. As expected, the potential binding between CD and maleic acid was very low. This demonstrated how difficult it is to make assumptions regarding the mechanism, based on the modest effects of the buffers shown by ITC alone.

Meta-analysis of effect of buffers on CD complexes

By making a meta-analysis of the effect of buffers on γ-CD, β-CD, HP-β-CD, and SBE-β-CD complexes [16, 17], it was evident that differences among the CDs were observed based on the size of the CD cavity (Fig. 6). Both citric and maleic acid decreased the stability of a β-CD complex by 57–65% [16] compared to > 15% for γ-CD. The difference in buffer effect on the β- and γ-CD complex was most likely related to the difference in cavity size between the two CDs. Most molecules can interact with the cavity of more than one CD, though there is a preference for one due to the size of the cavity [28]. Previous comparative studies showed that the guest adamantane carboxylic acid is too large to fit into α-CD, it fits snugly inside the β-CD, and it is too small to fill out the cavity of γ-CD [29, 30]. Adamantane carboxylic acid

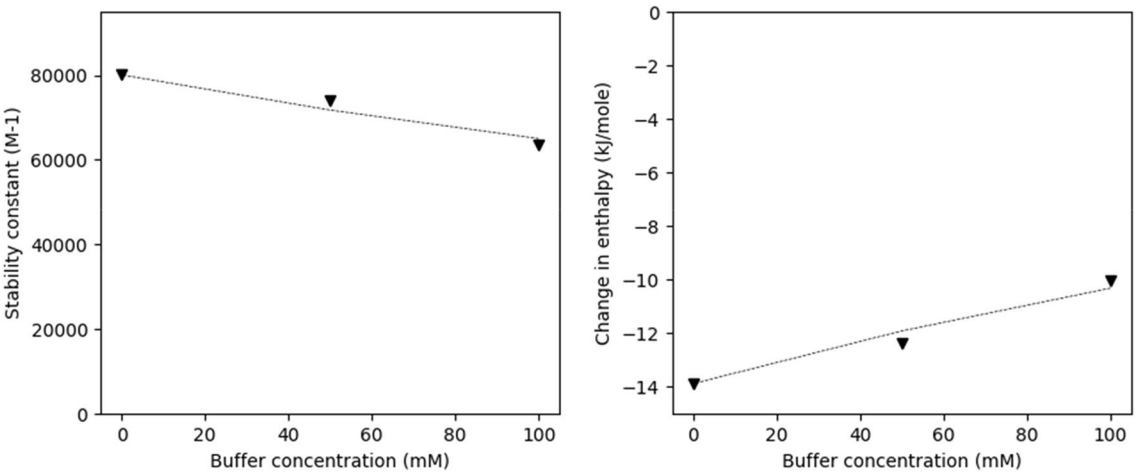


Fig. 5 Fitting curve for binding constant and change in enthalpy for γ-CD:TCDC in maleic acid based on the theoretical expressions for competitive interactions (Eqs. 1 and 2)

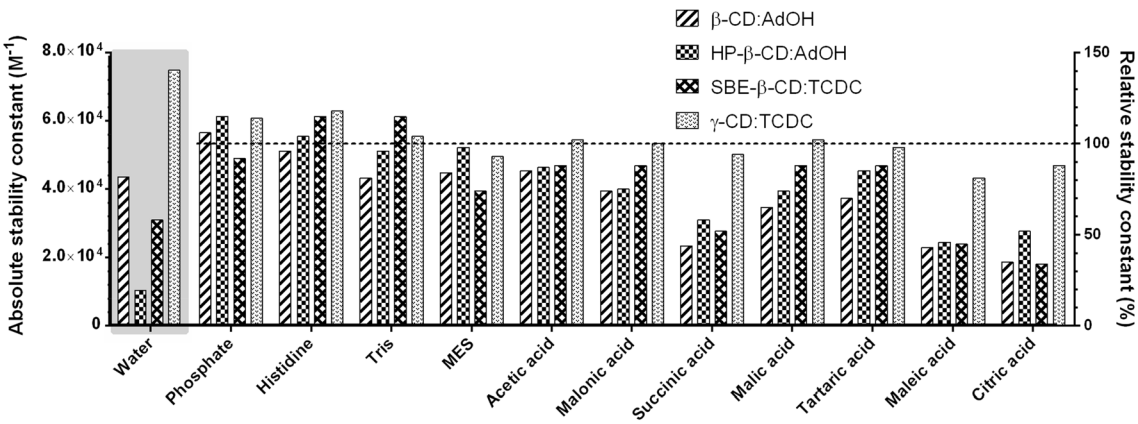


Fig. 6 Comparison of the relative effect (%) of 11 buffers on the absolute stability constants (M^{-1}) of β-CD:adamantanol (AdOH), HP-β-CD:AdOH, SBE-β-CD:TCDC, and γ-CD:TCDC, respectively. The figure combines data from Fig. 1, Samuelsen et al. [16], and Samuelsen et al. [17]

has a radius of about 7 Å and volume of about 180 Å, which is the optimal geometry for β-CD cavity, and an optimum match between CD cavity and guest results in the strongest binding [29]. Likewise, the size of the CD cavity also explains the difference in affinity for TCDC and the various CDs. TCDC showed a very weak affinity for the small cavity of α-CD [23], the highest affinity for β-CD around 10^5 M^{-1} [31], and medium affinity of approximately 8×10^4 M^{-1} for the γ-CD cavity [2]. Hence, the cavity relative to the guest size may serve as an explanation of the strong effect of citric acid and maleic acid on the apparent binding of the β-CD complex compared to the results from the γ-CD complex, i.e. the size of the β-CD cavity has the optimal size for the two buffers. Interestingly, of the three buffers, which affected the γ-CD:TCDC complex, maleic acid is the smallest, yet it showed the largest effect. One possible explanation is the rigidity of maleic acid due to the double bond in the carbon chain, compared to the more flexible structures of citric acid and MES. This explanation also accounts for the observation that smaller buffers, e.g. acetic acid, has a less pronounced effect on the CD complexes, as they are expected to be too small to fill out the cavities.

The cavity relative to the guest size hypothesis is further supported by the fact that carboxylic acid buffers have similar effect on β-CD, HP-β-CD and SBE-β-CD complexes [16, 17]. The natural and modified CDs have very different exteriors, and thus the results support that the effect of buffers is related to the size of the cavity rather than the exterior of the CD.

In contrast to the effect of carboxylic acid buffers, the MES buffer showed approximately 15% decrease in the stability of a β-CD complex [16], which is similar to the effect in this study on the γ-CD complex. MES is a bulky and large molecule, and a greater fit to the γ-CD cavity would be a reasonable assumption, though the results did not show this. MES showed no effect on a HP-β-CD complex [16], and the effect of MES on a SBE-β-CD complex was attributed to the ionic strength of the buffer rather than an interaction between MES and CD [17]. Hence, the presence of MES buffer decreased the stability of natural CD complexes, but not of CD derivative complexes. It is possible that MES interacts with the exterior of the CDs, i.e. formation of non-inclusion complexes, rather than the CD cavity, but it is not possible to directly to elude anything about the interaction mechanism of MES without further studies.

Inclusion complexes or non-inclusion complexes?

The results from the meta-analysis indicate interaction between carboxylic acid buffers and the β-CD cavity, which support formation of regular inclusion complexes between β-CD and buffers. It is difficult unambiguously to prove the existence of these inclusion complexes, since their stability constants are estimated to be very weak. Typically, NMR is considered a fundamental tool in characterization of inclusion complexes in solution and their conformation [32, 33]. Previous NMR results were unable to conclusively show formation of inclusion complexes between β-CD and buffers [16]. Though indications were there for inclusion of citric acid, the results were more doubtful for maleic and succinic acid, where very weak cross peaks was observed in combination with small changes in the chemical shift of the CD protons in ^{1}H NMR spectra [16]. However, the results did not show evidence of binding to the exterior of the CD either.

In the case of γ-CD and maleic acid, a traditional NMR titration is not suitable for the determination of the binding constant due to the low binding constant between the two. If the binding of maleic acid is 2 M^{-1}, as suggested above, it would require 2 M maleic acid for γ-CD to reach a saturation degree of 80%, which is necessary for optimal analysis. Though maleic acid has a high solubility in water, it is unwise to use concentrated solutions for the NMR titration, as the ionisc strength and solution properties will be different compared to diluted conditions.

Besides, one should be careful with using NMR spectroscopy alone to derive complex three-dimensional structures [33]. Some of the challenges with interpreting NMR spectra of CD complexes are the small shielding of protons in the CD cavity, i.e. in some cases the CD protons are only shifted by a few tenths of a ppm, and the strength of ROESY cross peaks depends on the intermolecular distance between protons and rapid exchange between binding modes [33]. The transient nature of some CD interactions has been demonstrated in a study, where routine NMR spectra did not show clear signs of interaction between β-CD and 5-flourouracil, however, it is suggested that it was due to a short lifetime of the complex estimated to approximately 13.5 ms [34]. It would make sense that the interactions between CDs and buffers have a transient nature, since the buffers may occur in different inclusion modes with CDs, and also, they must be expected to have somewhat favorable interactions with the surrounding water molecules due to their hydrophilicity. The transient nature of CD and buffer interactions and the poor saturation degree may explain why it has been difficult to prove inclusion formation between CD and buffers by NMR. On the other hand, one study argues that they have unambiguously proved formation of inclusion complexes between maleic, tartaric and fumaric acid and β-CD [11]. Though they observe small changes in chemical shift and weak cross peaks in their ROESY spectra, their conclusions are supported by a combination of NMR, infrared spectrometry and molecular modelling techniques. So, why was one study able to prove formation of inclusion complexes between β-CD and maleic acid, while another could not? This question remains unanswered, but evidence is there that

regular inclusion formation is possible between carboxylic acid buffers and CDs.

Conclusions

The apparent stability constant of the γ-CD:TCDC complex was affected by buffer species. Three of the eleven buffers (MES, maleic and citric acid) decreased the stability of the complex slightly, though the effect of the buffers had no practical relevance. By comparing the results with available data for buffer's effect on β-CD, HP-β-CD and SBE-β-CD complexes, it was found that the results support the formation of regular inclusion complexes for carboxylic acid buffers, where the affinity between buffer and CD is governed by the size of the CD cavity rather than the exterior of the CD.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s10847-021-01111-4.

Author contributions LS: Conceptualization, formal analysis, investigation, original draft. RH: Review and editing. CS: Review and editing.

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Data availability The dataset used and/or analyzed during the current study are available from any of the authors on reasonable request.

Declarations

Conflict of interest The authors have no competing interests.

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