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Thermodynamics and Structural Aspects of Propofol Inclusion in Native and Substituted β-Cyclodextrins in Water

Andrea Usenik,* Stella Katovčić, Josip Požar#

University of Zagreb Faculty of Science, Department of Chemistry, Horvatovac 102a, HR-10000, Zagreb, Croatia

* Corresponding author's e-mail address: ausenik.chem@pmf.hr

Corresponding author's e-mail address: pozar@chem.pmf.hr

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Abstract: Propofol (2,6-diisopropylphenol) is a widely used intravenous general anesthetic whose extremely low aqueous solubility requires lipid-emulsion formulations associated with pain on injection and further adverse effects. This problem could be overcome using biocompatible solubilizing excipients such as cyclodextrins. Here we report a detailed thermodynamic characterization of propofol inclusion within β-cyclodextrin and three pharmaceutically relevant derivatives: randomly methylated β-cyclodextrin, 2-hydroxypropyl-β-cyclodextrin, and sulfobutylether-β-cyclodextrin at 298 K. For this purpose, standard complex stability constants, reaction Gibbs energies, enthalpies, and entropies were determined by means of UV spectrophotometric, microcalorimetric, and ^{1}H NMR titrations, where the latter also offered structural insights into the complexes formed. All investigated cyclodextrins bind propofol with moderate and comparable affinity (log $K°$ = 2.7–3.3), however the substitution on the cyclodextrin rims markedly impacts the enthalpic and entropic contributions to standard complexation Gibbs energy. Pronounced temperature dependence of $\Delta_r H°$ and $T\Delta_r S°$ resulting in enthalpy-entropy compensation was observed in the 5–37 °C range for propofol/**β-CD** and propofol/**RM-β-CD** systems. This finding outlines the inadequacy of linear van't Hoff plots for enthalpy and entropy determination simultaneously demonstrating that the cyclodextrins efficiently bind propofol in the pharmaceutically relevant temperature window.

Keywords: propofol, cyclodextrin, inclusion complex, isothermal titration calorimetry, enthalpy-entropy compensation, hydrophobic effect.

INTRODUCTION

PROPOFOL (2,6-diisopropylphenol, Figure 1), is a phenol derivative bearing two isopropyl groups in the *ortho*-positions. It is one of the most widely used intravenous anesthetics, acting as a positive allosteric modulator of the $GABA_A$ receptor.[1] Propofol is, however, extremely lipophilic (octanol/water partition coefficient of the order of 10^4)[2] and by pharmaceutical standards virtually insoluble in water (sub-millimolar concentrations[3]), so it cannot be administered as an aqueous formulation. It is instead marketed as an oil-in-water lipid emulsion - and was originally formulated in the surfactant Cremophor EL carrying well-documented drawbacks: pain on injection ascribed largely to the free propofol in the aqueous phase reported in around 60 % of patients, susceptibility to microbial contamination, hypertriglyceridemia, and the risk of propofol-infusion syndrome on prolonged administration.[4,5] The mentioned adverse effects and formulation limitations have driven the search for lipid-free or lipid-reduced alternatives involving microemulsions and nanoemulsions, water-soluble prodrugs such as fospropofol, and most relevant genuine aqueous solutions based on solubilizing excipients.[4,6,7]

The potential of cyclodextrins as stabilizers and solubilizers of pharmaceutically active ingredients was first

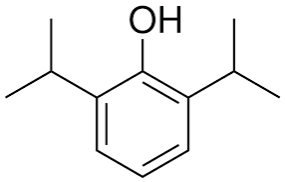

Figure 1. Molecular structure of propofol.

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suggested by Cramer in the 1950s.[8] However, the reports on their biotoxicity, probably a result of sample contamination, postponed their widespread usage for almost two decades.[9] Nowadays, these macrocyclic hosts built from glucopyranose units linked by α-(1→4) glycosidic bonds, formed as products of the enzymatic degradation of starch, belong to a class of most frequently used excipients in liquid and in solid formulations.[10–13] Apart from that, they are exploited in food, cosmetic, and chemical industry.[9,14–17]

The three most common and best studied natural homologues comprise six (α-cyclodextrin, **α-CD**), seven (β-cyclodextrin, **β-CD**), and eight (γ-cyclodextrin, **γ-CD**) glucose subunits.[9,14,18] These receptors adopt the shape of a truncated *cone* with less polar interior (the H-3 and H-5 hydrogen atoms point into the cavity), whereas the secondary (C-2, C-3) and primary (C-6) hydroxyl groups line the portals and the outer, more polar, water-exposed surface. Systematic studies of their host-guest chemistry began in the mid-twentieth century,[9,18] and following the onset of their industrial production, the thermodynamics of complexation of a large number of guests by cyclodextrins was characterized in detail, resulting in an extensively documented field of supramolecular chemistry.[9,14,18–21] In the majority of cases complexes of 1:1 stoichiometry are formed, the stability of which depends on the host-guest size compatibility, the guest saturation degree, its shape and functionalization, as well as on ionic strength and on pH (for guest with pH-dependent speciation).[9,18,22] Considering the compiled stability constants for native cyclodextrins, **α-CD** preferentially binds small linear hydrocarbons, the cavity of **β-CD** is well suited to monocyclic and bicyclic derivatives (propofol included), and **γ-CD** readily accommodates larger guests such as diamantanes and steroids.[18,23–25] The experimental methods used for stability constant determination most frequently include UV-Vis and NMR spectroscopy, fluorimetry, and more recently, isothermal titration calorimetry. The latter technique is particularly informative as it allows the complete characterization of the binding event (i.e. the determination of the equilibrium constant and associated standard reaction enthalpy, hence also $\Delta_r G°$ and $\Delta_r S°$) providing detailed insight into the thermodynamics governing the inclusion.

The frequently exothermic nature of inclusion into cyclodextrins (**α-CD** and **β-CD** in particular) contrasts with the thermodynamics of association of apolar species in water (e.g. micellization[26,27]), which is entropy-driven (classical hydrophobic effect).[28–31] The discrepancy is, in the case of classically hydrated guests, rationalized by the release of "frustrated" cavity water (non-classical hydrophobic effect).[29,32,33] Namely, the water molecules confined inside a less polar cavity form fewer hydrogen bonds than in the bulk, so their liberation upon guest binding is an enthalpically favorable process, strongly contributing to the overall complex stability.[29,33] The magnitude of this complexation driving force depends on the number of included water molecules and the hydrogen bonding patterns realized among them, and hence on the cavity size, providing a unifying picture for the binding of hydrophobic guests by cyclodextrins, cucurbiturils, and related synthetic receptors.[19,23,25,29,33,34]

The pharmaceutical potential of **β-CD** as a solubilizer and stabilizer of lipophilic active pharmaceutical ingredients is limited by its own relatively low aqueous solubility (lower than that of **α-CD** or **γ-CD**) which is attributed to intramolecular hydrogen bonding between adjacent secondary hydroxyl groups and intermolecular interactions between cyclodextrin molecules in the crystal form.[11,13] Functionalization of the portal hydroxyl groups disrupts this network. Hydroxypropyl, methyl, and sulfobutylether derivatives (Figure 2) are roughly two orders of magnitude more soluble than the parent **β-CD**, and their complexes with active pharmaceutical ingredients often exhibit superior bioavailability and stability relative to the free drug.[11,13,35–39] Substituted cyclodextrins are also biologically well tolerated, being ultimately metabolized to simple sugars. The anionic sulfobutylether-β-cyclodextrin (**SB-β-CD**, marketed as Captisol®), in particular, is non-nephrotoxic and hence used in numerous approved parenteral formulations.[35] Concerning the cyclodextrin-anesthetic formulations, the

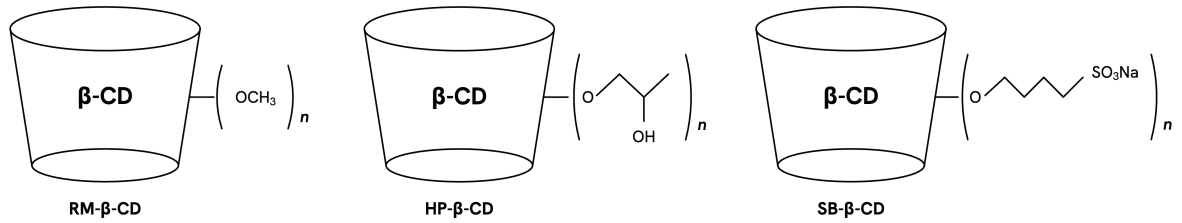

Figure 2. Structures of the studied β-cyclodextrin derivatives: randomly methylated β-cyclodextrin (**RM-β-CD**), 2-hydroxypropyl-β-cyclodextrin (**HP-β-CD**), and sulfobutylether-β-cyclodextrin (**SB-β-CD**); *n* denotes the average number of substituted cyclodextrin OH groups (average degree of substitution, DS).

neurosteroid alfaxalone (intrinsic aqueous solubility below 5 μg mL^{-1}) is marketed as a lipid- and surfactant-free aqueous solution in **HP-β-CD** (Alfaxan), in which inclusion raises its apparent solubility by orders of magnitude and removes the histamine release provoked by the earlier castor-oil/surfactant formulation.[40–42] For propofol itself, inclusion complexes with **HP-β-CD** and **SB-β-CD** increase its apparent aqueous solubility, can reproduce the pharmacokinetic profile of the lipid emulsion, and have been evaluated in humans for transport across the blood-brain barrier.[3,7,43–46]

Despite the potential application interest, the complexation of propofol by cyclodextrins has so far been characterized almost exclusively by the phase-solubility method of Higuchi and Connors,[47] which delivers the apparent complex stability constant.[48] Its binding with **HP-β-CD** in deionized water and in 0.05 mol dm^{-3} phosphate buffer (pH = 6.5) was characterized by apparent stability constants of log K ≈ 3.60 and log K ≈ 3.54 respectively.[3,43] Interestingly, the **SB-β-CD** exhibited rather similar affinity for propofol (log K ≈ 3.57, phosphate buffer (pH = 7)).[44] In the two instances where reaction enthalpies and entropies for the inclusion within mentioned cyclodextrins were also given, they were not obtained directly but extracted from the temperature dependence of the phase-solubility constant assuming that $\Delta_r H°$ and $\Delta_r S°$ remain constant in the explored temperature range. As the binding of non-polar guest by cyclodextrins is accompanied by a particularly negative reaction heat capacity, giving rise to pronounced temperature enthalpy-entropy compensation,[23,25,29,33,49] this is most likely unjustified. Further, the indirectly determined standard thermodynamic binding parameters are quite different: complexation with **HP-β-CD** was reported as enthalpy-driven and entropically opposed ($\Delta_r H°$ = −26.7 kJ mol^{-1}, $\Delta_r S°$ = −22 J K^{-1} mol^{-1}),[43] whereas that with **SB-β-CD** was found to be entropy-driven and isoenthalpic ($\Delta_r H°$ = −1.2 kJ mol^{-1}, $\Delta_r S°$ = 65 J K^{-1} mol^{-1}).[44] To the best of our knowledge, the inclusion of propofol within **β-CD** or with randomly methylated **β-CD** has not been examined so far. Likewise, no reports concerning the ITC studies of propofol complexation with natural or derivatized seven-membered cyclodextrins can be found in the literature. Apart from obvious application interest, such comparative studies of the receptor functionalization impact on the complex stability and underlying binding thermodynamics offer valuable insights into the cavity hydration and the guest to host size-affinity relationship, especially when accompanied by spectroscopic investigations of the complexes.

Here we report the thermodynamic characterization of the inclusion of propofol within **β-CD**, 2-hydroxypropyl-**β-CD** (**HP-β-CD**), randomly methylated **β-CD** (**RM-β-CD**) and sulfobutylether-**β-CD** (**SB-β-CD**) in water at 298 K aiming to assess how the type and degree of rim substitution affect the complex stability and influence the enthalpic and entropic contributions to the standard reaction Gibbs energy. To achieve this goal, we relied on ITC to derive a full set of standard binding parameters, checking the reliability of obtained equilibrium constants by means of UV spectrometric titrations. The insights into the guest inclusion depth and complex geometry were provided by ^{1}H NMR spectroscopy. We also examined the temperature influence on the stability of propofol complexes with **β-CD** and **RM-β-CD** and the related standard thermodynamic reaction quantities. The results offer interesting information concerning the cyclodextrin functionalization impact on the binding thermodynamics simultaneously allowing the evaluation of cyclodextrins comprised of seven monomeric subunits as propofol excipients in pharmaceutically and physiologically relevant temperature window.

EXPERIMENTAL

Materials

Propofol (Sigma-Aldrich, 97 %) was used as received. β-cyclodextrin (**β-CD**; Sigma-Aldrich, ≥ 98 %), randomly methylated β-cyclodextrin (**RM-β-CD**; Cyclolab, > 95 %, DS ≈ 12), 2-hydroxypropyl-β-cyclodextrin (**HP-β-CD**, Sigma-Aldrich, ≥ 98 %, DS ≈ 5.6), and sulfobutylether-β-cyclodextrin (**SB-β-CD**; Cyclolab, > 95 %, DS ≈ 6) were dried at 150 °C for 3 h prior to use. The degrees of substitution (DS) quoted by the suppliers refer to the average total number of substituents per cyclodextrin ring, which can also be expressed per glucose ring, where it amounts to roughly 0.80 (**HP-β-CD**), 1.7 (**RM-β-CD**), and 0.86 (**SB-β-CD**).

Propofol solutions were prepared by weighing the neat liquid, adding a sufficient amount of Milli-Q water (for UV and ITC experiments) or D_2O (Sigma-Aldrich, > 99.9 % D; for NMR experiments) to achieve concentration below solubility, followed by brief sonication, and finally dilution in a volumetric flask. Concentration of the propofol solution used for spectrophotometric and microcalorimetric measurements was 1.37 × 10^{-4} mol dm^{-3} and 4.0 × 10^{-4} mol dm^{-3} for ^{1}H NMR titrations. It is worth noting that the concentrations of propofol solutions were kept far below its solubility (≈150 μg cm^{-3} (ref. [3]), which corresponds to s ≈ 8 × 10^{-4} mol dm^{-3}) and no solubilizers were added (e.g. cosolvents). Concentrations of cyclodextrin solutions were kept between 1.0 × 10^{-2} and 2.0 × 10^{-2} mol dm^{-3}.

UV Spectrophotometric Titrations

UV spectra were recorded on an Agilent Cary 60 spectrophotometer in a quartz cell (l = 1 cm) at 298 K. The

receptor solution was added directly into the cell containing the propofol solution (V_0 = 2.2 cm^3), and spectra were corrected for dilution. Each titration was repeated three or more times. Stability constants and the characteristic molar absorption spectra of the complexes were obtained by non-linear least-squares regression of the absorbance data assuming 1:1 complex formation using the program HypSpec.[50]

Isothermal Titration Calorimetry

Calorimetric measurements were carried out on a MicroCal VP-ITC instrument at 298 K. The receptor solution was added from a 300 µL syringe into the reaction cell (V = 1.45 mL) containing the propofol solution. Each titration was repeated three or more times. Non-linear regression analysis of the thermograms was carried out with the complex stoichiometry fixed at N = 1 (one set of sites model, MicroCal Origin 7.0), to yield the stability constant, reaction enthalpy, and, by combination, the reaction Gibbs energy and entropy. Fitting of the calorimetric data using a sequential two-site model accounting for a complex of higher stoichiometry (either **G$_2$H** or **GH$_2$**) did not improve the agreement between the fitted and the experimental values, and the simplest 1:1 model was retained.

^{1}H NMR Spectroscopy

^{1}H NMR spectra were recorded on a Bruker Avance 400 MHz spectrometer in D_2O at 298 K. Spectra of propofol were first recorded in the presence of an excess of each receptor to identify the protons whose chemical shifts respond to complexation. Subsequently, ^{1}H NMR titrations were performed by adding receptor solution to a propofol solution. Complex stability constants were obtained by non-linear least-squares regression of the chemical shift data assuming 1:1 complex formation using the program HypNMR.[51]

RESULTS AND DISCUSSION

Stability Constants and Reaction Thermodynamics

The inclusion of propofol at 298 K was first examined by UV spectrophotometric titration with **β-CD** (Figure 3) and the three derivatives (Figures S1–3). Upon successive addition of receptor, systematic changes in the propofol absorption band were observed; the dependence of the absorbance at 278–280 nm on the receptor-to-guest mole ratio is consistent with the formation of a stable 1:1 complex in every case. Regression analysis yields stability constants of the order log $K°$ ≈ 3 (Table 1), indicating that all four receptors bind propofol appreciably in water. The thermograms and the normalized successive enthalpy changes (Figures 4 and S4–6) were also satisfactorily described by a 1:1 binding model, giving stability constants in good agreement with the spectrophotometric values and enabling direct determination of the reaction enthalpy, thus resulting in a complete thermodynamic binding profile ($\Delta_rG°$, $\Delta_rH°$, and $\Delta_rS°$, Table 1). The stability constants determined by NMR experiments (Figures 5 and S7–9, Table 1) were also in good agreement with those obtained by the two other methods.

Formation of the complexes of propofol with **β-CD** and its derivatives at 298 K is both enthalpically and entropically favorable. The stability constants vary modestly across the series (log $K°$ from 2.7 to 3.3, i.e. $\Delta_rG°$ between about −16 and −19 kJ mol^{-1}), so that the choice of substituent has a rather limited effect on the overall affinity, with slightly larger affinity of **RM-β-CD** compared to other hosts. In contrast, the enthalpic and entropic contributions vary substantially from system to system. For native **β-CD** the reaction is markedly enthalpy-favored ($\Delta_rH°$ = −9.14 kJ mol^{-1}), whereas for **HP-β-CD** and **RM-β-CD**

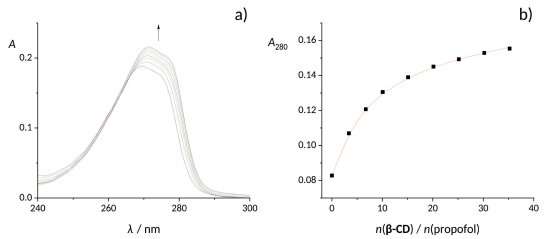

Figure 3. Representative UV spectrophotometric titration of propofol (c_0 = 1.37 × 10^{-4} mol dm^{-3}) with **β-CD** (c = 1 × 10^{-2} mol dm^{-3}) in H_2O at 298 K; V_0(propofol) = 2.2 cm^3; l = 1 cm. a) Spectra corrected for dilution. b) Dependence of the absorbance at 280 nm on the receptor-to-guest molar ratio. ■ experimental; – calculated.

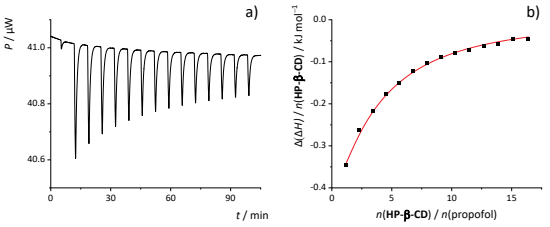

Figure 4. Microcalorimetric titration of propofol (c_0 = 1.37 × 10^{-4} mol dm^{-3}) with **HP-β-CD** (c = 1.10 × 10^{-2} mol dm^{-3}) in H_2O at 298 K. a) Thermogram. b) Dependence of the normalized successive enthalpy change on the receptor-to-guest molar ratio. ■ experimental; – calculated.

Table 1. Thermodynamic parameters for the complexation of propofol with **β-CD** and its derivatives in H_2O at 298 K, determined by UV spectrophotometric titrations, ITC and ^{1}H NMR.[(a)]

Receptor	log *K*°	$\Delta_r G°$ / kJ mol^{-1}	$\Delta_r H°$ / kJ mol^{-1}	$-T\Delta_r S°$ / kJ mol^{-1}
β-CD	2.89(1) ^{ITC} 2.86(4) ^{UV} 2.98 ^{NMR}	−16.10(4)	−9.14(1)	−7.02(2)
RM-β-CD	3.31(2) ^{ITC} 3.23(1) ^{UV} 3.36 ^{NMR}	−18.9(1)	−2.09(3)	−16.8(1)
HP-β-CD	2.97(2) ^{ITC} 3.01(3) ^{UV} 3.30 ^{NMR}	−17.0(1)	−3.46(2)	−13.5(3)
SB-β-CD	3.12(8) ^{ITC} 2.73(2) ^{UV} 3.33 ^{NMR}	−17.8(4)	−7.4(2)	−10.4(3)

[(a)] The standard error of the mean is given in parentheses as the uncertainty in the last significant figure.

the binding enthalpy is close to zero (−3.46 and −2.09 kJ mol^{-1}, respectively) hence essentially entropically driven ($-T\Delta_r S°$ = −13.5 and −16.8 kJ mol^{-1}, respectively). With this respect, the agreement between the UV, NMR and ITC derived stability constants backs the reliability of calorimetrically determined complexation parameters. As far as the binding thermodynamics is concerned, the observed enthalpy-entropy compensation for the examined receptor series is consistent with the larger apolar surface of more highly substituted receptors, which enhances the classical, entropy-driven hydrophobic contribution of the guest dehydration, simultaneously diminishing the enthalpic gain associated with the release of "frustrated" cavity water.[52–55] This rationale is corroborated by ^{1}H NMR spectroscopy (Figures 5, S7–9). Addition of an excess of each receptor shifts the aromatic protons of propofol to lower chemical shifts (the effect is most pronounced for **HP-β-CD**), particularly those in the *para*-position with respect to the hydroxyl group indicating deep penetration of the aromatic ring into the cavity. Conversely, the opposite shift of the propofol methyl protons is consistent with their location near the polar, hydroxyl-bearing rim. On the other hand, the downfield shift of isopropyl protons upon the **HP-β-CD** (Figure 5) and **SB-β-CD** addition (Figure S9) may reflect a stronger influence of the larger, electronegative or anionic groups at the CD rims. Significant changes can also be observed in the splitting pattern of the CH_3 signals. While in non-bound propofol the two isopropyl methyls are enantiotopic (single doublet in ^{1}H NMR), upon its inclusion in the chiral cavity they become diastereotopic, resulting in doublet splitting into two doublets (in analogy to the γ-CH_3 protons of valine, Figure S9). Upon further addition of **RM-β-CD** (Figure S8) these two doublets even overlap into an apparent triplet.

The progression in the values of the binding parameters (Figure 6) can be traced to the appended groups themselves. Namely, the 2-hydroxypropyl, methyl, and sulfobutylether substituents extend and loosen the cyclodextrin rim, so that inclusion of propofol is accompanied by dehydration of the substituents in addition to that of the guest. Release of this additional ordered water surrounding the guest and, presumably, the non-polar substituents strengthen the entropic driving force, while the greater flexibility and the more open structure of the extended receptor cavity reduce the enthalpic gain that dominates binding to the more rigid native **β-CD**. The two contributions largely compensate, so that rim functionalization reshapes the enthalpic and entropic terms while leaving the overall affinity almost unchanged.

The effect of cyclodextrin rim substitution on the thermodynamic complexation parameters can depend more on which hydroxyl groups (primary O-6 at the narrower rim or secondary O-2/O-3 at the wider rim) are functionalized than on the substitution degree. Among methylated CDs, substitution at O-6 has a positive effect on the receptors' affinity due to elongation of the cavity, however the opposite holds when secondary OH groups are functionalized due to disruption of the intramolecular hydrogen bonds and the steric hindrance for guest inclusion introduced by functional groups at O-3, oriented towards the cavity.[56] For randomly methylated **β-CD** the distribution among all OH groups is almost statistical,[57] suggesting that part of the mixture with methylated O-3 groups has lower affinity compared to the native **β-CD**, while substitution on other OH groups increases the binding affinity. According to our data (Table 1), the increase in affinity upon methylation could suggest larger contribution of the CDs functionalized at O-2 and O-6 compared to O-3. Data on the hydroxypropylation pattern

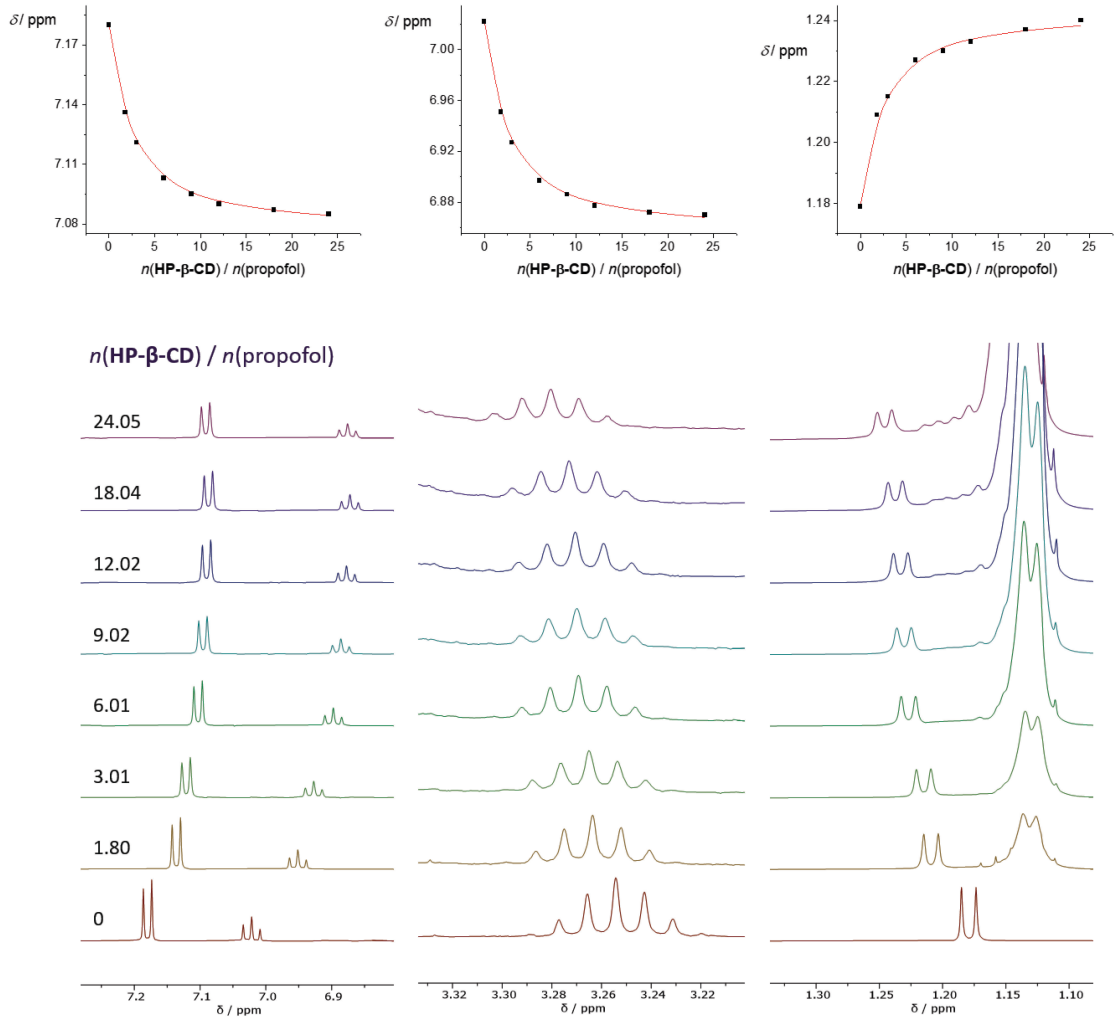

Figure 5. ^{1}H NMR titration of propofol (c_0 = 4 × 10^{-4} mol dm^{-3}) with **HP-β-CD** (c = 2 × 10^{-2} mol dm^{-3}) in D_2O at 298 K.

of **HP-β-CD** is known for commercially available Cavasol® W7 HP, where the functionalization is directed mainly to O-2 (58 %), while a significantly smaller amount of the substituents reside on O-3 (32 %) and O-6 (10 %).[58] Sulfobutylether groups can even be preferentially bound to O-3 (50 %) with only 40 % at O-2 and 10 % at O-6,[59] or can be randomly distributed.[60] Bearing in mind that the position of the substituents largely depends on the receptor used, the average stability constant does not follow the degree of substitution monotonically. In native **β-CD** intramolecular O3−H···O2′ hydrogen bonds around the secondary rim keep the receptor rigid. Substitution interrupts this pattern and the modified hosts tend to have a more flexible, open, and extended cavity.[56] This added flexibility, together with the smaller amount of cavity water, lowers the enthalpic gain on binding and is

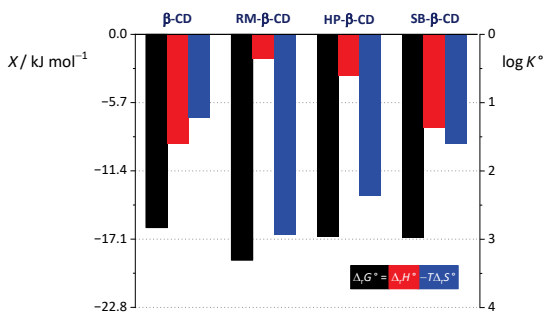

Figure 6. Enthalpic and entropic contributions to the standard reaction Gibbs energy for the complexation of propofol with **β-CD**, **RM-β-CD**, **HP-β-CD**, and **SB-β-CD** in H_2O at 298 K.

consistent with the shift toward entropically driven inclusion for the more heavily substituted receptors.

In the end it should be mentioned that near-invariance of $\Delta_r G°$ alongside the pronounced variation of $\Delta_r H°$ and $-T\Delta_r S°$ is a textbook manifestation of enthalpy-entropy compensation, in which a change in one term is counterbalanced by an opposite change in the other.[18,24,61,62] Such compensation is characteristic of host-guest equilibria dominated by reorganization of water, and it is most pronounced for the **β-CD** and **RM-β-CD** pair, followed by **β-CD** and **HP-β-CD**. It can be concluded that the extension of the receptor's less polar cavity gradually shifts the inclusion thermodynamic fingerprint from both enthalpy (non-classical hydrophobic effect) and entropy driven towards essentially entropy driven (classical hydrophobic effect). This could be ascribed to the increase in flexibility of the cavity upon functionalization, rendering the cavity water more bulk-like and its dehydration less exothermic, but also to additional dehydration of the classically hydrated substituents on the cyclodextrin rims.[63–65]

In order to evaluate the temperature-induced changes in the hydration of the reactants and the resulting complexes, we have investigated the complexation of propofol by **β-CD** (Figures S10 and S11) and **RM-β-CD** (Figures S12 and S13) in a pharmaceutically relevant temperature range (5–37 °C). The results presented in Table 2 and Figure 7 show significant temperature dependence of $\Delta_r H°$ and $-T\Delta_r S°$, with both the standard reaction enthalpy and entropy decreasing with T. This in turn results in temperature-induced enthalpy-entropy compensation and very low variation of complex stability ($\Delta_r G°$) with temperature, a characteristic of cyclodextrin complexation reactions.[23,25,49,54,66–68]

When the herein presented results are compared against the handful of data available for propofol-cyclodextrin systems, all of which were obtained by the phase-solubility method,[3,43,44] two features stand out. First, the apparent stability constants derived from phase solubility (log $K°$ ≈ 3.5–3.6) are systematically larger than the values determined here by two independent techniques (log $K°$ ≈ 2.9–3.0). This is consistent with the well-documented sensitivity of phase-solubility analysis when applied to a guest of very low intrinsic solubility, such as propofol. In the context of potential application of cyclodextrins as propofol carriers, the fraction of propofol that remains free in the aqueous phase, which governs both the onset of anesthesia and the injection pain, is defined by the stability constant and the cyclodextrin excess. Tellingly, a lipid-free **SB-β-CD** solution of propofol was found in a human study not to reduce, and even to increase, injection pain relative to the conventional lipid emulsion, an outcome consistent with a substantial free-drug concentration in solution.[7] This obstacle can be overcome by adding the receptor in sufficient excess once the associated equilibrium constant has been reliably evaluated.

Second, the enthalpies and entropies reported in the literature were obtained via the van't Hoff expression, assuming the temperature-independence of binding enthalpy and entropy ($\Delta_r C_p° = 0$). As mentioned in the Introduction, the hydrophobic effect is characterized by strongly negative reaction heat capacities leading to notable compensation of $\Delta_r H°$ and $-T\Delta_r S°$ contributions to standard reaction Gibbs energy over a wide temperature range. The effect is primarily caused by the temperature-induced "melting" of guest hydration spheres, and is, besides its fundamental implications, important for cyclodextrin application in water, as it reveals quite weak variation of its binding affinity with temperature. With this respect, the reported van't Hoff analysis assigns the **HP-β-CD** complex an enthalpy-driven, entropy-opposed formation ($\Delta_r H° = -26.7$ kJ mol^{-1}, $\Delta_r S° < 0$) indicating a strong temperature dependence of stability constant,[43]

Table 2. Temperature dependence of thermodynamic parameters for the complexation of propofol with **β-CD** and **RM-β-CD** in H_2O determined by ITC.[a]

Receptor	T / K	log $K°$	$\Delta_r G°$ / kJ mol^{-1}	$\Delta_r H°$ / kJ mol^{-1}	$-T\Delta_r S°$ / kJ mol^{-1}	$\Delta_r C_p°$ / J K^{-1} mol^{-1} [b]
β-CD	278	3.07(1)	−16.34(8)	−3.22(5)	−13.1(1)	−297(4)
	298	2.89(1)	−16.10(4)	−9.14(1)	−7.02(2)	
	310	2.85(1)	−16.71(4)	−13.0(2)	−3.7(3)	
RM-β-CD	278	3.18(4)	−16.9(2)	3.3(4)	−20.2(2)	−219(22)
	298	3.31(2)	−18.9(1)	−2.09(3)	−16.8(1)	
	310	3.30(2)	−19.6(1)	−4.6(1)	−15.0(2)	

[a] The standard error of the mean is given in parentheses as the uncertainty in the last significant figure.

[b] Uncertainties of the last digit(s) are given in parentheses as standard deviations obtained by weighted linear regression analysis.

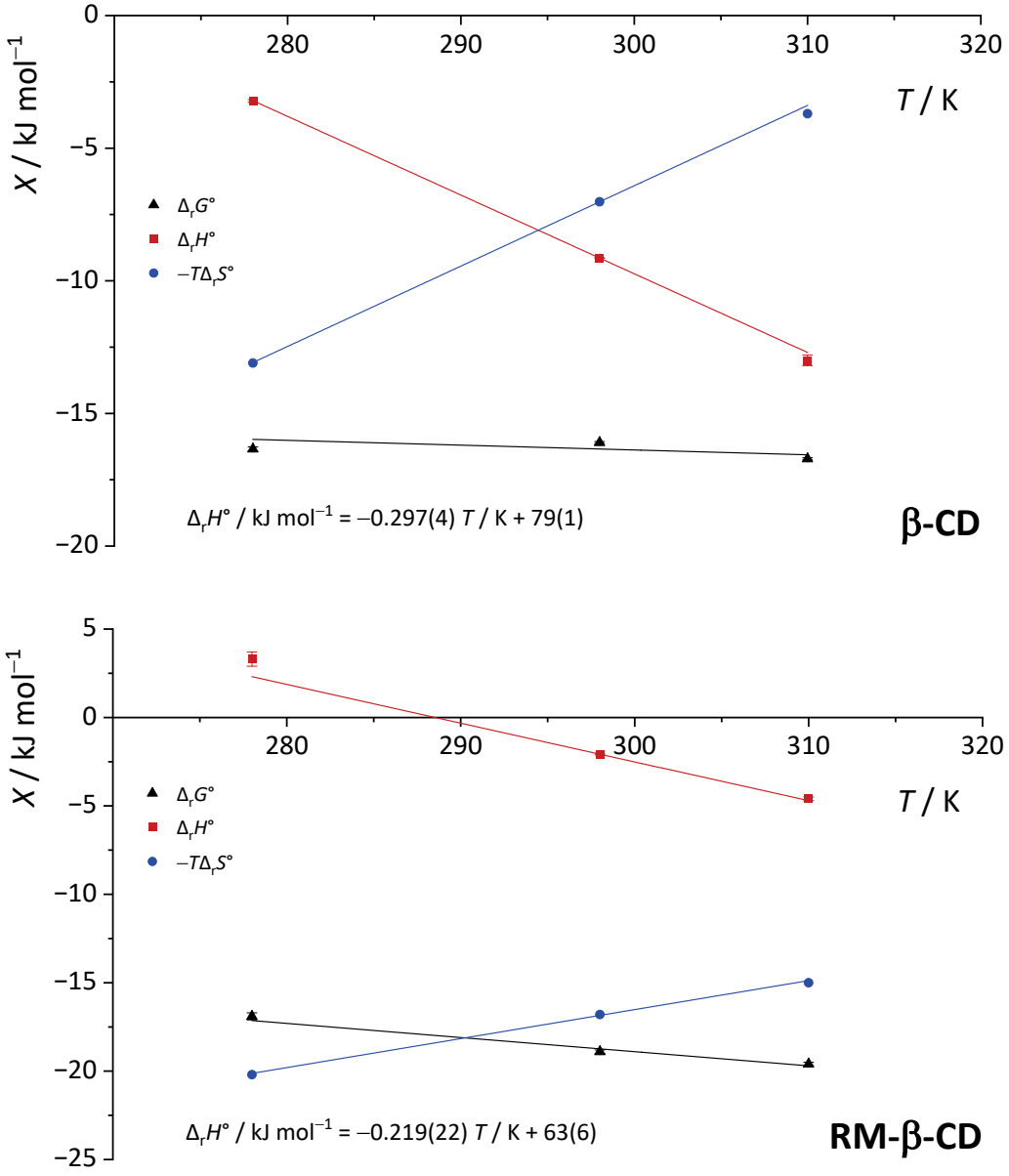

Figure 7. The temperature dependence of standard complexation parameters of propofol with **β-CD** (above) and **RM-β-CD** (below) in H_2O.

whereas direct calorimetry reveals a small favorable enthalpy and a dominant favorable entropy ($\Delta_r H°$ = −3.46 kJ mol^{-1}, $-T\Delta_r S°$ = −13.5 kJ mol^{-1}). The herein performed calorimetric titrations (5–37 °C) on propofol-**β-CD** and propofol-**RM-β-CD** systems reveal strongly negative reaction heat capacities leading to weak temperature dependence of $\Delta_r G°$, implying that the hosts comprised of seven monomeric subunits bind propofol with comparable affinity over a pharmaceutically relevant temperature range.

CONCLUSION

The inclusion of the intravenous anesthetic propofol by native β-cyclodextrin and by its hydroxypropyl, randomly methylated, and sulfobutylether derivatives was characterized thermodynamically in water at 298 K by UV spectrophotometric and isothermal titration calorimetric titrations, relying on ^{1}H NMR spectroscopy for structural insights. All four receptors form 1:1 inclusion complexes of moderate and comparable stability (log $K°$ ≈ 2.7–3.3),

demonstrating that highly soluble β-cyclodextrin derivatives retain an affinity for propofol comparable to that of the parent macrocycle, supporting their use as propofol-solubilizing excipients. While the affinity is more or less insensitive to the nature of the substituent, the enthalpic and entropic contributions vary strongly and compensate one another: the more highly substituted **HP-β-CD** and **RM-β-CD** bind propofol in an increasingly entropy-driven fashion (classical hydrophobic effect), consistent with their larger apolar surface. By contrast, the complexation with native **β-CD** bears the fingerprint of non-classical hydrophobic effect (predominantly enthalpy driven complexation). These trends reflect a gradual change between the enthalpy- and entropy-dominated limits of the hydrophobic effect. Further, the ^{1}H NMR data place the aromatic ring of the guest deep within the cavity, leaving the isopropyl and hydroxyl group partially solvent exposed. The microcalorimetric titrations performed in a wider temperature range revealed that the inclusion is characterized by strongly negative reaction heat capacities which also lead to notable enthalpy-entropy compensation with temperature, indicating that the pharmaceutical potential of cyclodextrins as propofol solubilizers and stabilizers is retained from 5 up to 37 °C.

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Supplementary Information. Supporting information to the paper is attached to the electronic version of the article at: https://doi.org/10.5562/cca4394.

PDF files with attached documents are best viewed with Adobe Acrobat Reader which is free and can be downloaded from Adobe's web site.

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