“Exploring 3D structural influences of aliphatic and aromatic chemicals on α -cyclodextrin binding”

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17 **Abstract:**

18 *Binding of solutes to macromolecules is often influenced by steric effects caused by the 3D*
19 *structures of both binding partners. In this study, the 1:1 α -cyclodextrin (α CD) binding*
20 *constants (K_{a1}) for 70 organic chemicals were determined to explore the solute-structural*
21 *effects on the α CD binding. K_{a1} was measured using a three-part partitioning system with*
22 *either a headspace or a passive sampler serving as the reference phase. The K_{a1} values*
23 *ranged from 1.08 to 4.97 log units. The results show that longer linear aliphatic chemicals*
24 *form more stable complexes than shorter ones, and that the position of the functional*
25 *group has a strong influence on K_{a1} , even stronger than the type of the functional group.*
26 *Comparison of linear and variously branched aliphatic chemicals indicates that having a*
27 *sterically unhindered alkyl chain is favorable for binding. These results suggest that only*
28 *one alkyl chain can enter the binding cavity. Relatively small aromatic chemicals such as*
29 *1,3-dichlorobenzene bind to α CD well, while larger ones like tetrachlorobenzene and 3-ring*
30 *aromatic chemicals show only a weak interaction with α CD, which can be explained by*
31 *cavity exclusion. The findings of this study help interpret cyclodextrin binding data and*
32 *facilitate the understanding of binding processes to macromolecules.*

33 **Keywords:**

34 *α -Cyclodextrin (CD); Steric effect; Binding constant; Inclusion complex;*
35 *Cyclodextrin water partitioning; solute 3D structure;*

36

37 1. Introduction:

38 *The binding of small molecules to macromolecules is important in numerous processes*
39 *such as enzymatic reactions, receptor binding, plasma protein binding, and drug*
40 *formulation with excipients. While partitioning of small molecules between various*
41 *homogenous phases such as solvents are well understood and quantified according to the*
42 *contributions of specific molecular interactions [1], this is yet not the case for the binding*
43 *to macromolecules. In contrast to homogeneous partitioning systems, the influence of the*
44 *three-dimensional (3D) structure plays a decisive role in the sorption process to*
45 *macromolecules. Generally, a good fit between the small molecule and the macromolecule*
46 *is important for the efficiency of the binding process [2].*

47 *An example of macromolecules that are used to bind smaller chemicals are cyclodextrins*
48 *(CDs). CDs are conic ring oligosaccharides and are also present naturally. CDs are usually*
49 *made of 6, 7, and 8 glucopyranose units, which are named α -, β -, and γ -CD, respectively.*
50 *The conic ring structure of CD generates a cavity. Its surface is mostly formed by the*
51 *hydrophobic parts of the molecule [3]. The molecular structure of CDs can also be modified*
52 *to increase their particular applicability, e.g., six modified CDs are widely used as*
53 *excipients for clinical purposes [4]. Here, one advantage of CD as excipient is its low*
54 *toxicity [4]; orally applied, CDs have shown low absorption to the blood circulation and*
55 *therefore exerted no toxic effect [5].*

56 *In solution, CDs commonly form inclusion complexes (host-guest complexes) with many*
57 *chemicals. Typically studied guests are drugs whose molecular mass ranges from 100 to*
58 *400 Da [6]. The specificity of CD binding appears to be relatively low, and the association*
59 *constants ($K [M^{-1}]$) vary widely across different guest molecules: for example, protonated*

60 aniline has a log K of 0.36 for the association with α -CD [7] while decyltrimethylammonium
61 bromide has a log K of 3.57 [8], and nucleotides can have $\log K \geq 6$ for the association with
62 aminocyclodextrins [7]. While CDs are sometimes considered like a normal, homogeneous
63 phase [9], the 3D structure of the small molecules appears to play a critical role for the
64 formation of the host-guest complex with CD [10] as for other macromolecular binding.

65 Energetics associated with the formation of the host-guest complex with CD are discussed
66 in the literature based on two concepts: a) Direct intermolecular interactions between host
67 and guest via van der Waals forces and hydrogen bonding which are influenced by the fit
68 between the guest and the CD cavity, and b) additional positive energy gains through the
69 formation of the host-guest complex. The latter includes mechanisms such as: the release
70 of bound water from the cavity to bulk water, and the relief of conformational stress of the
71 cyclodextrin [11]. The relative importance of the different factors on the partition process
72 should depend on the guest molecule.

73 In addition to the beneficial use for clinical and other industrial purposes, CD is often
74 considered a model macromolecule to study host-guest complexation. An advantage of
75 using CD for studying molecular steric effects on binding behavior is its well-investigated
76 3D structure. The binding is flexible to some degree but restricted in the conic main
77 structure [12]. The angles between the glucopyranose units vary depending on the
78 solvation medium, the host-guest complex, and the aggregate state. Binding coefficients
79 of CD give direct indications of the strength of binding, but experimental data found in the
80 literature (e.g., [10, 13]) are derived from many sources that use different methods. Thus,
81 the data are not always comparable and the composition of the data set might not be
82 designed to answer specific questions regarding the influence of the 3D structure.

83 *In this study we experimentally determined a large, consistent dataset of binding*
84 *constants for α CD with 70 aliphatic and aromatic chemicals such as alcohols, ethers and*
85 *chlorobenzenes. The aim of this study was to identify the 3D-structural features of guest*
86 *molecules that influence the binding affinity to α CD. Particularly, we sought for*
87 *explanations for substantially different binding constants that we found for apparently*
88 *similar chemicals.*

89 **2. Materials and methods**

90 **2.1 Materials**

91 *The chemicals were purchased from various providers and their purity was at least 94 %*
92 *and mostly >98 %, as listed in the supporting information (SI). There were some chiral*
93 *chemicals, the chirality of which was not specified. All test chemicals used for binding*
94 *experiments were first dissolved in methanol to make stock solutions. Three to five*
95 *chemicals of one compound class were mixed into one stock solution. Only those chemicals*
96 *that were distinctly separated through the gas-chromatographic (GC) system (see below)*
97 *were mixed together. The concentration of each chemical in methanol stock solution did*
98 *not exceed 10 % of the water solubility so that the final concentration after dilution in*
99 *water was well below the solubility limit. For all experiments pure water produced by a*
100 *MilliQ Gradient A10 system (Millipore) was used. Polyacrylate (PA, coating thickness 36*
101 *μ m, volume of the coating 16.5 μ L/m)- and poly(dimethylsiloxane) (PDMS, coating*
102 *thickness 30 μ m, volume of the coating 13.2 μ L/m)-coated glass fibers produced by*
103 *Polymicro Technologies Inc. (Phoenix, AZ) were purchased from Optronics GmbH (Kehl,*
104 *Germany). α CD was obtained from Wacker Chemie AG with a purity of at least 98.0 % and*
105 *a maximum residual complexant (1-decanol) of 20 ppm. α CD (0.5 to 2 g) was weighed into*

106 a 100 mL volumetric flask and dissolved with MilliQ water to prepare CD stock solution,
107 which was diluted further before the binding experiment.

108 2.2 Instruments

109 The following equipment was used for the quantitative analysis: Hewlett Packard GC
110 System HP 6890 series gas-chromatographs with a flame ionization detector (FID) or an
111 electron capture detector (ECD), both systems connected to an HP 7694 Headspace
112 Sampler; an Agilent 7890A GC System equipped with a 5975C inert MSD Triple Axis
113 Detector and a Gerstel Multi Purpose Sampler (MPS 2XL). The chemicals were analyzed on
114 either of the following two columns from Agilent Technologies: HP-1 (30 m × 0.32 mm i.d.,
115 4 µm film thickness), or HP-5MS (30 m × 0.25 mm i.d., 0.25 µm film thickness).

116 2.3 Binding experiments

117 Binding constants for 70 chemicals were measured in batch systems. The used methods
118 have been described in detail previously [14] and are briefly explained below. In both
119 methods, the unbound, freely dissolved concentration of the chemical was determined via
120 the measurement of a third phase, either air (headspace approach) or a PA or PDMS fiber
121 (passive sampling approach). All binding experiments were performed at 30 °C, which was
122 the lowest possible temperature that the sample tray of the GC autosampler was able to
123 control.

124 2.3.1 Headspace approach

125 Air was the common third phase (reference phase) for this approach [15]. Two groups of
126 weighed 20 mL vials were prepared with four vials per group. One group was filled with 5
127 mL water and the other was filled with 5 mL αCD solution (2 - 15 g/L). The vials were

spiked with 10 or 25 μL of methanolic stock solution of the selected chemicals and were immediately closed with a PTFE- or aluminum-lined silicone septum to prevent loss of the chemicals. From the experience of preliminary experiments, the equilibrium time was set to a minimum of four hours: first three hours on a horizontal shaker at 30 °C with 300 rpm and then at least one hour on the GC-sample tray at 30 °C with low shaking speed. Then the headspace was probed with a 100 μL sampling loop or a 250 μL syringe and injected into the GC and measured with GC-FID/ECD or GC-MS.

2.3.2 Passive sampling approach

The passive sampling approach was used for chemicals which are not volatile enough for the headspace approach. PA or PDMS fiber is the common reference phase for this approach [16, 17]. The experimental setting was similar to that with the headspace approach except for the following changes. The volume of the solutions and the vials was 10 mL, each vial received 5 or 10 cm of PA- or PDMS-coated fiber and the equilibrium time was 72 hours at 30 °C. Previous studies [18, 19] confirmed that this equilibrium time is sufficient for a wide range of chemicals. After equilibrium was reached, the PA/PDMS fibers were removed from the vials and carefully wiped with a clean tissue. Then the fibers were extracted overnight on a roll mixer using 200 μL of cyclohexane (for PDMS) or ethyl acetate (for PA). The concentrations of the extract were quantified with the GC-MS system using external calibration.

2.4 Data analysis

The results of GC analyses were evaluated using the same approach as Geisler et al [14]. Thus, the partition coefficient between the αCD solution and water ($K_{\text{CD solution/water}}$ [$L_{\text{water}}/L_{\text{CD solution}}$]) was determined from the relative GC peak areas of vials with and

151 without α CD. In cases where partitioning of the chemical into air or the fiber contributed
 152 substantially to the mass balance, this was considered in the calculation of $K_{CD\ solution/water}$
 153 based on known air-water or fiber-water partition coefficient of the chemical [14]. The
 154 resulting $K_{CD\ solution/water}$ was used to derive the partition coefficient between α CD and water
 155 ($K_{CD/water} [L_{water}/kg_{CD}]$) according to:

$$K_{CD\ solution/water} = K_{CD/water} c_{CD} + f_{water} \quad (1)$$

156 c_{CD} is the concentration of α CD in the α CD solution [$kg_{CD}/L_{CD\ solution}$] and f_{water} is the volume
 157 fraction of water in the α CD solution [$L_{water}/L_{CD\ solution}$]. As c_{CD} was only up to 0.015 kg/L in
 158 this work, f_{water} was considered unity.

159 2.5 Stoichiometry of the complexes

160 The stoichiometry of the CD host-guest complexes can differ depending on the types and
 161 concentrations of CD and guest [20-22]. The common stoichiometry is 1:1 and 2:1 but other
 162 kinds of complexes can be found in the literature [8, 23-27]. Previous studies show that
 163 smaller chemicals tend to form only 1:1 complexes, while larger ones can also form 2:1
 164 complexes [28]. For studying interactions of CD with various guest molecules, it is
 165 advantageous to compile consistent data for 1:1 complexes.

166 Complexation constants for 1:1 and 2:1 binding can be expressed as,

$$K_{a1} = \frac{[S\alpha CD]}{[S][\alpha CD]} \quad (2)$$

$$K_{a2} = \frac{[S(\alpha CD)_2]}{[S\alpha CD][\alpha CD]} \quad (3)$$

$$K_{a1}K_{a2} = \frac{[S(\alpha CD)_2]}{[S][\alpha CD]^2} \quad (4)$$

167 Where K_{a1} and K_{a2} [M^{-1}] are the formation constants for the 1:1 and 2:1 complexes,
 168 respectively. S is the substrate (guest), and $S\alpha CD$ and $S(\alpha CD)_2$ are the 1:1 and 2:1
 169 complexes, respectively. Assuming that 1:1 and 2:1 binding is dominant, the partition
 170 coefficient introduced in eq. 1 has the following relationship with the binding constants:

$$K_{CD \text{ solution/water}} = f_{\text{water}} (K_{a1}[\alpha CD] + K_{a1}K_{a2}[\alpha CD]^2 + 1) \quad (5)$$

171 If K_{a2} is very small, then the second term in the parentheses and therefore 2:1 binding
 172 become negligible and $K_{CD \text{ solution/water}}$ depends linearly on the concentration of αCD . This
 173 implies that a linear relationship between $K_{CD \text{ solution/water}}$ and $[\alpha CD]$ indicates a dominance
 174 of 1:1 binding. The relative importance of 2:1 binding becomes higher with higher $[\alpha CD]$,
 175 as indicated by the squared concentration in the second term of eq. 5. We experimentally
 176 tested the influence of the αCD concentration (0.5, 1, 5, 10, 15, 20 g/L) on $K_{CD \text{ solution/water}}$ for
 177 chemicals with a long linear alkyl chain (i.e., 6-undecanone, 2-undecanone, 5-decanone, 2-
 178 decanone, dihexylether, dipentylether, dibutylether), because they are more likely to form
 179 2:1 complexes with αCD than their shorter analogues [29]. Also, we tested the influence of
 180 $[\alpha CD]$ on the binding of three aromatic chemicals of different sizes (chlorobenzene, 1,3-
 181 dichlorobenzene, pentachlorobenzene) to ensure 1:1 binding. Equation 5 was fitted to
 182 these concentration dependent data for $K_{CD \text{ solution/water}}$ to obtain K_{a1} and K_{a2} . For the other
 183 chemicals, single concentration data for $K_{CD \text{ solution/water}}$ were measured and used to derive
 184 K_{a1} , assuming no significant 2:1 binding (i.e., $K_{a2} = 0$ in eq 5).

3. Results and discussion

3.1 Measurements of K_{a1}

The concentration dependent measurements (see figures in SI) show that $K_{CD \text{ solution/water}}$ increases linearly with $[CD]$ for chlorobenzene, 1,3-dichlorobenzene, and pentachlorobenzene, suggesting dominant 1:1 binding. For 6-undecanone, 2-undecanone, dihexylether, dipentylether, dibutylether, 5-decanone, and 2-decanone, the relationship was nonlinear to varying degree. Nevertheless, these chemicals also form mostly 1:1 complexes if the αCD concentration is smaller than 5 g/L (i.e., 5 mM), as suggested by eq 5 in combination with K_{a1} and K_{a2} obtained by fitting. At $[CD] \leq 2 \text{ mM}$, the second term in eq 5 has only a small contribution, and K_{a1} could also be derived from a single concentration measurement with an error below 0.15 log units. For these reasons, we conclude that it is valid to assume a dominance of 1:1 binding for the studied aromatic chemicals in the $[CD]$ range we used and for the tested aliphatic chemicals in the low $[CD]$ range, which allows the use of equation 5 with $K_{a2} = 0$ to derive K_{a1} . This assumption is consistent with the literature, where it is reported that short chain surfactants (C8) do not form 2:1 complexes to a considerable degree [30] and that long chain surfactants (C10 and C12) form mostly 1:1 complexes with some contributions from 2:1 complexes [8, 31, 32].

Table 1 K_{a1} and K_{a2} derived from the concentration dependent measurement

Chemical	$K_{a1} [M^{-1}]$		Standard error		$K_{a2} [M^{-1}]$		Standard error	
6-undecanone	8.13	10^2	0.24	10^2	7.6	10^1	0.5	10^1
2-undecanone	6.31	10^3	0.46	10^3	9.7	10^1	1.8	10^1
dihexylether	2.55	10^3	0.28	10^3	1.28	10^2	0.27	10^2
dipentylether	4.65	10^2	0.61	10^2	1.10	10^2	0.25	10^2
dibutylether	2.09	10^2	0.19	10^2	4.4	10^1	1.4	10^1
5-decanone	5.24	10^2	0.62	10^2	4.5	10^1	1.6	10^1
2-decanone	3.86	10^3	0.03	10^3	6.7	10^1	0.2	10^1
chlorobenzene	1.25	10^2	0.24	10^2	0.9	10^1	1.5	10^1

1,3-dichlorobenzene	4.69	10 ²	0.14	10 ²	0.6	10 ¹	0.2	10 ¹
pentachlorobenzene	1.2	10 ¹	0.4	10 ¹	0			

203

204 The K_{a1} values of 70 chemicals were determined in batch experiments. The chemical set
 205 comprises: 19 alcohols, 19 ketones, 9 polycyclic aromatic hydrocarbons (PAHs), 6
 206 chlorobenzenes, 5 alkylbenzenes, 4 ethers, 4 nitroalkanes, and 4
 207 phosphates/phosphonates. These chemicals have various functional groups but relatively
 208 simple molecular structures, which should facilitate interpretation of the results.
 209 Moreover, the data set includes multiple series of chemicals with increasing number of
 210 structural units (i.e., -CH₂-, Cl-, aromatic ring), enabling the assessment of incremental
 211 effects on the binding behavior. We avoided measuring complex chemicals like drugs,
 212 because they have many different chemical features which could cause indistinguishable
 213 effects on the binding to α CD. The measured $\log K_{a1}$ values span over a wide range, from
 214 1.08 (pentachlorobenzene) to 4.97 (1-dodecanol). For 10 chemicals, K_{a1} was too small to
 215 measure with the applied method (denoted with $\log K_{a1} < 1.3$ in Table 2).

216 **Table 2 Experimental α CD/water binding coefficients of the measured chemicals.**

Class	Chemical	$\log K_{a1}$	SD or SE
End-substituted alcohols	1-Butanol	1.64	0.09
	1-Pentanol	2.22	0.04
	1-Hexanol	2.63	0.03
	1-Heptanol	3.08	0.04
	1-Octanol	3.29	0.08
	1-Nonanol	3.83	0.21
	1-Decanol	4.32	0.06
	1-Undecanol	4.72	0.06
	1-Dodecanol	4.97	0.07
Middle-substituted alcohols	3-Hexanol	1.83	0.07
	4-Heptanol	2.16	0.05
	4-Octanol	2.35	0.04
	5-Nonanol	2.48	0.04
	5-Decanol	2.86	0.12

Branched alcohols	2-Methyl-2-propanol	< 1.3	
	3-Ethyl-3-pentanol	1.62	0.11
	2-Ethyl-1-hexanol	2.82	0.06
	3-Ethyl-3-hexanol	2.21	0.05
	4-Ethyl-3-hexanol	2.10	0.05
End-substituted ketones	2-Pentanone	1.50	0.02
	2-Hexanone	2.01	0.01
	2-Heptanone	2.46	0.01
	2-Octanone	2.89	0.01
	2-Nonanone	3.26	0.06
	2-Decanone ^a	3.59	0.01
	2-Undecanone ^a	3.80	0.03
Middle-substituted ketones	3-Pentanone	< 1.3	
	3-Hexanone	1.71	0.08
	4-Heptanone	2.05	0.06
	4-Octanone	2.35	0.05
	5-Nonanone	2.55	0.06
	5-Decanone ^a	2.72	0.05
	6-Undecanone ^a	2.91	0.01
Branched and cyclic ketones	3-Methyl-2-butanone	< 1.3	
	4-Methyl-2-pentanone	1.31	0.09
	Cyclopentanone	< 1.3	
	Cyclohexanone	< 1.3	
	Cycloheptanone	1.36	0.38
Nitroalkanes	Nitroethane	< 1.3	
	1-Nitropropane	1.49	0.05
	1-Nitrobutane	1.94	0.03
	1-Nitrohexane	2.68	0.04
Ethers	Dipropyl ether	1.88	0.31
	Dibutyl ether ^a	2.32	0.04
	Dipentyl ether ^a	2.67	0.05
	Dihexyl ether ^a	3.41	0.04
Trialkyl phosphates	Triethyl phosphate	1.69	0.15
	Tri-n-propyl phosphate	1.88	0.09
	Tri-n-butyl phosphate	2.30	0.07
	Diethyl ethylphosphonate	2.01	0.17
Alkylbenzenes	Toluene	1.68	0.05
	Ethylbenzene	2.28	0.02
	n-Propylbenzene	2.93	0.01
	n-Butylbenzene	3.08	0.05
	n-Hexylbenzene	3.48	0.13
Chlorobenzenes	Chlorobenzene ^a	2.10	0.08

PAHs	1,3-Dichlorobenzene ^a	2.67	0.01
	1,2,4-Trichlorobenzene	2.19	0.03
	1,2,4,5-Tetrachlorobenzene	1.25	0.07
	Pentachlorobenzene ^a	1.08	0.12
	Hexachlorobenzene	1.40	0.26
	Naphthalene	1.59	0.07
	Acenaphthylene	< 1.3	
	Acenaphthene	< 1.3	
	Biphenyl	1.67	0.16
	1-Chloronaphthalene	< 1.3	
	Fluorene	1.85	0.25
	Phenanthrene	< 1.3	
	Dibenzothiophene	2.79	0.10
	Fluoranthene	1.69	0.21

217 ^a Data are derived from the concentration dependent measurement. The error presented is
218 SE for these data. For the other data, the error shown is SD.

219 3.2 Correlation with the octanol-water partition coefficient (K_{OW})

220 The log octanol-water partition coefficient (K_{OW}) is often related to log $K_{CD/water}$ [33-36] and
221 was even proposed as a descriptor for predictions [34]. The log K_{a1} values measured in this
222 study are compared to log K_{OW} in Figure 1. The experimental log K_{OW} values were taken
223 from the EPIsuite 4.1 database [37] if available, and otherwise, KOWWIN was used to
224 estimate log K_{OW} .

225

226 *Figure 1 Experimental 1:1 α CD binding coefficients versus octanol/water partition*
 227 *coefficients. The $\log K_{a1}$ values were determined at 30 °C and the $\log K_{OW}$ values were from*
 228 *the EPIsuite 4.1 database or predicted with KOWWIN. The white circles indicate chemicals*
 229 *with $\log K_{a1} < 1.3$. The solid line indicates the linear regression of the black circles.*

230 *As shown in Figure 1, the correlation between $\log K_{OW}$ and $\log K_{a1}$ is weak, with R^2 of 0.19.*
 231 *Correlation is particularly weak in the high K_{OW} range (i.e., $\log K_{OW} > 3$). For example, 1-*
 232 *dodecanol and pentachlorobenzene have similar $\log K_{OW}$ values (5.13 and 5.17,*
 233 *respectively) but differ more than 3 log units in their K_{a1} values (4.96 and 1.08,*
 234 *respectively). Conversely, nitroethane and phenanthrene have > 4 log units different log*
 235 *K_{OW} values (0.18 and 4.46, respectively) but $\log K_{a1}$ values are both < 1.3. As has been*
 236 *demonstrated by others, the correlation between $\log K_{OW}$ and $\log K_{CD/water}$ can be higher if*
 237 *only one chemical class is considered [35, 38]. However, $\log K_{OW}$ is not useful for the*
 238 *understanding of the specific binding process to α CD, nor for estimating $\log K_{CD/water}$ if*

different classes are considered. Obviously, $\log K_{OW}$ does not account for the 3D structure effects. Therefore, in the following sections, we analyze the data further and try to identify the specific steric factors influencing the binding process to α CD.

3.3 Aliphatic compounds

The experimentally derived binding coefficients of chemicals with one or more linear alkyl chains (14 alcohols, 14 ketones, 4 nitroalkanes, 4 ethers, and 3 phosphates) are plotted in log units against the number of carbon atoms (Figure 2). More specifically, we include here (1) linear aliphatic compounds with the polar functional group at the end of the molecule, i.e., $R-OH$, $R-C(=O)CH_3$, and $R-NO_2$, where R is a linear alkyl chain of differing lengths, (2) aliphatic compounds with the polar functional group in the middle of the molecule, i.e., $R-C(OH)-R'$, $R-C(=O)-R'$, and $R-O-R$, where $R' = R$ or $R-CH_2-$ (i.e., one unit longer), and (3) trialkyl phosphates (i.e., PO_4-RRR). The $\log K_{a1}$ values within each homologous group increase linearly with the number of carbon atoms. It is remarkable that $\log K_{a1}$ of n -alkan-1-ols ($R-OH$) increase linearly from 1-butanol to 1-decanol. The torus of α CD has a height of 8 Å [11], whereas the distance between the atomic nuclei of C1 and C10 of decanol is around 11.5 Å for the stretched conformer [39]. Theoretically, around 3 Å of the stretched 1-decanol molecule should stick out of the α CD cavity and experience water as the surrounding phase, from which there is no energy gain to be expected for the sorption process. Thus, the linear increase of $\log K_{a1}$ for n -alkan-1-ols suggests that the alkyl chain or α CD—or both—adapt their conformation, which enables optimal interactions between the alkyl chain and α CD for molecules that are too long to fit into the cavity in their stretched conformation. Comparable data have been published for surfactants (alkyltrimethylammonium bromide/chloride) in regard to the chain length; K_{a1} values

larger than 10^4 M^{-1} are reported for 12 carbon atoms [8]. The K_{a1} values of 1-undecanol and 1-dodecanol measured in this study are even higher than that of 1-decanol but the stoichiometry might not be solely 1:1, as the concentration dependent measurements in this study do not cover chemicals with a linear alkyl chain length > 9 . We were not able to perform these experiments with 1-undecanol and 1-dodecanol because the GC peak shapes were too distorted. A similar trend for alcohols has been reported also in the literature (see SI) but with 1-nonanol as the largest chemical.

Figure 2 Experimental K_{a1} for α CD versus number of carbon atoms. The lines indicate the linear regressions. The dashed lines are the regressions for end- and middle-substituted alcohols which are extrapolated to the intersection. The grey lines are the regressions for end- and middle-substituted ketones which are extrapolated to the intersection. The data points for ethanol and acetone were derived by extrapolation and have not been measured.

277 Chemicals with the functional group at the end of the molecule have generally higher K_{a1}
278 than chemicals with the same functional group in the middle, when compared at the same
279 number of C (Figure 2). Moreover, the slopes of the three end-substituted chemical classes
280 are similar, and so are the slopes of the three middle-substituted classes. However, there is
281 a substantial difference in the slopes between end-substituted and middle-substituted
282 classes (0.40 and 0.26 log units/C on average, respectively). That means that elongation of
283 the molecule in one direction increases K_{a1} more than elongation in two directions, per
284 carbon atom. Trialkyl phosphates (elongation in three directions) exhibit an even smaller
285 slope (0.1 log units/C). Such a differential increase per C does not occur with solvent-water
286 partition coefficients such as K_{OW} and thus has to be caused by steric effects.

287 The observations above are consistent with the concept available in the literature [40, 41]
288 that the polar functional group of the bound guest molecule stays outside the hydrophobic
289 cavity and interacts with the surrounding water or with one of the hydroxyl groups of the
290 CD rims. Thus, the polar functional group restricts the location and the orientation of the
291 guest relative to CD and can thereby hinder the optimal interactions of the alkyl chain(s)
292 with the CD cavity. It is plausible that the polar functional group stays outside the cavity,
293 because the polar functional group of the free, unbound chemical can undergo strong
294 hydrogen bonding interactions with water molecules, whereas hydrogen bonds cannot be
295 formed inside the hydrophobic cavity of CD. Thus, the polar functional group could enter
296 the cavity only if that leads to a free energy gain that is larger than the free energy loss
297 due to the breakup of hydrogen bonds with water. Assuming that the polar functional
298 group has to be outside the cavity, end-substituted chemicals may still fully insert their
299 alkyl chain into the cavity, whereas middle-substituted chemicals may not insert both
300 chains well in the cavity.

301 *It is also worth noting that the regression lines for end- and middle-substituted chemicals*
302 *intersect at 2 and 3 carbon atoms for alcohols and ketones, respectively (Figure 2). These*
303 *intersections correspond to ethanol and acetone. This is reasonable, as the structural*
304 *difference between end- and middle substitutions diminishes with decreasing number of*
305 *carbon atoms.*

306 *To obtain more insights into the binding mechanisms of aliphatic compounds to α CD, we*
307 *estimated the number of carbon atoms within the cavity based on the following, very*
308 *simple assumptions: 1. The polar functional group cannot enter the cavity. 2. Only one*
309 *alkyl chain per molecule can enter the cavity, and the number of encapsulated carbon*
310 *atoms is not restricted by the height of the CD torus. 3. If there are two chains or more*
311 *with differing lengths, then the longest one enters the cavity (see Figure 3; only the*
312 *numbered carbon atoms are assumed to be in the cavity). In Figure 4, $\log K_{a1}$ is plotted*
313 *against the number of encapsulated carbon atoms estimated this way.*

314

315 *Figure 3 Scheme for estimating the number of encapsulated carbon atoms by the α CD*
 316 *cavity. The numbered carbon atoms are assumed to be in the CD cavity, which is*
 317 *represented as the dotted circle.*

Figure 4 Experimental K_{a1} versus estimated number of encapsulated carbon atoms. The lines indicate the linear regressions. The linear regressions for middle-substituted alcohols and ketones are omitted for clarity.

There are several findings in Figure 4. First, plots for end-functionalized alcohols, ketones and nitroalkanes overlap each other; that is, end-functionalized linear chemicals from different classes have similar $\log K_{a1}$ when the estimated number of carbon atoms in the cavity is identical. This shows that the functional group influences K_{a1} similarly for alcohols, ketones and nitroalkanes. If the functional group interacts with water in both bound and unbound states, its net energy contribution to the binding is close to zero, and thus the type of functional group has only a limited influence on K_{a1} , agreeing with the data in Figure 4. Note that the functional groups considered here are all polar and can form hydrogen bonds with water. Second, slopes and positions of middle-functionalized chemicals and trialkyl phosphates are now close to those of end-functionalized chemicals. Third, $\log K_{a1}$ of the middle-substituted chemicals are consistently higher than those of the

end-substituted chemicals when compared at the same number of estimated C. This shift is roughly between 0.2–0.5 log units and smaller than the increment per C for the end-functionalized chemicals. This supports the assumption that only one alkyl chain enters the cavity. The shift itself may be explained by some additional energy gain from the alkyl chain that is assumed outside the cavity or the carbon atom that is designated as C_x in Figure 4. Perhaps, these carbon atoms can still interact with the rim or the outside of the CD molecule. But energy gain from the non-encapsulated carbon atoms appears to be much less than that of the encapsulated ones.

It has to be repeated that the middle-substituted alcohols and ketones plotted in Figure 4 are not all symmetric but include those chemicals with one chain that is one CH₂ unit longer than the other chain. The middle-substituted alcohols and ketones do show linear increase depending on the encapsulated number of carbon atoms in the cavity but the data scatter more than end-substituted chemicals. This also suggests some interactions between the non-encapsulated alkyl group and αCD.

Overall, the concept that polar functional group stays outside the cavity and only one alkyl chain enters the cavity can explain the data trends of mono-functional, linear aliphatic chemicals very well, but additionally, αCD seems to interact with the parts of the guest molecule that are not considered encapsulated in the cavity.

3.4 Aliphatic chemicals with branched alkyl chain

We measured five alcohols with 8 carbon atoms: 1-octanol, 2-ethyl-1-hexanol, 4-octanol, 3-ethyl-3-hexanol, and 4-ethyl-3-hexanol. The log K_{a1} values decrease in this order (Figure 5) and also the length of the longest linear, non-branched alkyl chain within the molecule.

355 These data also imply a favorable interaction between α CD and a linear alkyl chain,
356 although not excluding possible interactions of the rest of the molecule with α CD.

357 While K_{a1} of a chemical with an ethyl-branched alkyl chain is lower than that of its non-
358 branched isomer, the energetic contribution of the additional ethyl group is always
359 positive. Hence, $\log K_{a1}$ is higher for 2-ethyl-1-hexanol (2.81) than for 1-hexanol (2.62), and
360 $\log K_{a1}$ of 3-ethyl-3-hexanol and 4-ethyl-3-hexanol is higher than that of 3-hexanol. It is
361 thus apparent that the branched ethyl group can also interact with CD and has a
362 significant contribution to K_{a1} .

363

364 *Figure 5 Experimental K_{a1} for α CD binding of 2 C_6 -alcohols and 5 C_8 -alcohols.*

365 3.5 Aromatic chemicals

366 The aromatic chemicals studied in this work are nine PAHs, six chlorobenzenes, and five
367 alkylbenzenes. The alkylbenzenes contain one linear alkyl chain of increasing length, but

368 $\log K_{a1}$ is not a simple linear function of the number of C atoms (Figure 6), in contrast to the
 369 polar aliphatic compounds shown above. The increment in $\log K_{a1}$ is 0.62 per additional
 370 methylene unit from toluene to propylbenzene, which is higher than the mean increment
 371 of 0.40 for the end-substituted linear aliphatic compounds. From propylbenzene to
 372 hexylbenzene the increment in $\log K_{a1}$ per additional methylene unit is 0.19. The benzene
 373 ring does not form a strong H-bond with water and thus can favorably enter the
 374 hydrophobic cavity of α CD. As the benzene ring occupies a fraction of the cavity,
 375 alkylbenzenes possessing an alkyl chain with three or more carbon atoms appear to
 376 experience a steric effect that lowers the $\log K_{a1}$ increase per C.

377

378 Figure 6 Experimental K_{a1} versus molecular weight for alkylbenzenes (circles) and
 379 chlorobenzenes (triangles). The molecular structures show the corresponding
 380 chlorobenzenes.

381 Chlorobenzenes represent an even more pronounced example of the influence of steric
382 restriction. The $\log K_{a1}$ values are above 2 for chemicals possessing one to three chlorine
383 atoms; 1,3-dichlorobenzene has the highest $\log K_{a1}$ (2.66), after which $\log K_{a1}$ starts to
384 decrease with an increasing number of chlorine atoms. The 1,2,4-trichlorobenzene has a
385 $\log K_{a1}$ (2.19) that is around 0.8 log units higher than the $\log K_{a1}$ of 1,2,4,5-
386 tetrachlorobenzene, pentachlorobenzene, and hexachlorobenzene. Mono and 1,3-
387 dichlorobenzenes appear to fit into the cavity, whereas 1,2,4-trichlorobenzene already
388 experiences a negative steric effect. The $\log K_{a1}$ for 1,2,4,5-tetrachlorobenzene is even
389 lower than that of monochlorobenzene, suggesting that the three additional chlorine-
390 substitutions hinder the interactions of the benzene ring and the original chlorine atom
391 with α CD.

392 PAHs also exhibit clear steric restriction. Toluene (1.68), biphenyl (1.67) and naphthalene
393 (1.59) have similar $\log K_{a1}$ values, even though the latter two molecules are much larger,
394 indicating that more than one aromatic six-ring cannot fully fit into the α CD cavity (Table
395 2). Moreover, naphthalene has a higher $\log K_{a1}$ value than 1-chloronaphthalene,
396 acenaphthene, acenaphthylene, and phenanthrene. This suggests that there is no room
397 left in the cavity for an element other than hydrogen around the naphthalene structure.

398 Dibenzothiophene, an aromatic three-ring system with a sulfur atom, exhibits the highest
399 $\log K_{a1}$ (2.79) of all PAH-related compounds tested. It is interesting that its $\log K_{a1}$ value is
400 0.94 log units higher than that of fluorene (1.85), the structural analogue of
401 dibenzothiophene with S being replaced by C. It is unknown why there is such a large
402 difference in K_{a1} between dibenzothiophene and fluorene. The 3D structure and the
403 electron distribution appear similar, as indicated by the sigma profiles and the COSMO files
404 generated by quantum chemical software Turbomole [44]. Our experimental observation is

consistent with the literature data for β CD binding constants of fluorene ($\log K_{\beta CD} = 3.08$ [42]) and dibenzothiophene ($\log K_{\beta CD} = 3.48$ [43]). The difference is smaller than that of α CD but the trend is similar. We also tried to measure the binding constants of pyrene, chrysene, benzo[a]pyrene and benzo[b]fluoranthene, but the difference between the partitioning in α CD solution and water was too small (pyrene) or concentrations in the PDMS fiber were below our detection limit.

4. Conclusions

In this study, we observed clear steric restrictions which influence the binding process to α CD. Particularly, hydrophobic aromatic chemicals indicated clear size limitations. The width of the α CD cavity may be represented by 1,3-dichlorobenzene or naphthalene, and any bulkier chemicals do not fit well into the cavity. Considering this clear size effect on aromatic compounds, it is surprising that linear aliphatic chemicals with an alkyl chain longer than nine carbon atoms still exhibit high binding coefficients. The question remains: how is the long alkyl chain able to fit into the cavity or how else is the high interaction energy explainable? There should be an adaption of the alkyl chain to the cavity as well as some other parts of the α CD. This would require some bending of the alkyl chain. Crystallographic data or molecular dynamics simulations would be needed to obtain a better insight into this phenomenon.

Another finding is that the binding of polar aliphatic chemicals strongly depends on the position of the functional group which restricts the length of the alkyl chain interacting with the α CD cavity. In general, a long unhindered alkyl chain appears to act as an anchor on a bigger molecule and should be able to bind to α CD. The general trends identified in this data set of organic chemicals should provide useful information for practical

428 applications of α CD. For example, high affinity of α CD for linear aliphatic compounds
429 relative to branched, inflexible compounds could be used for selective binding and
430 separation of these chemicals.

431 The experimental data of this work are highly consistent and diverse and thus are useful in
432 a quantitative modelling approach for $\log K_{a1}$. The simple correlation with $\log K_{ow}$ was
433 found to be too low to be useful for prediction purposes. Our results strongly indicate that
434 any modeling approach should consider the 3D structure of the α CD and the guest
435 molecule. We are currently working on the use of 3D quantitative structure activity
436 relationships for modeling of the binding data presented above, which will be reported in
437 an upcoming article.

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443

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