ORIGINAL ARTICLE

"Surface water" and "strong-bonded water" in cyclodextrins: a Karl Fischer titration approach

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Abstract Cyclodextrins are some of the most used carriers for bioactive compounds (as host–guest complex) and many factors influence the association–dissociation of this complex, some of them being related to hydrophobicity. In the solid state, cyclodextrins contain two types of water molecules: "surface" water molecules (especially close to the crystal surface) and "strong-bonded" water molecules (especially from the cyclodextrin cavity), but the classification is hard to do, and the concentration of these water molecules are relatively difficult to estimate by simple methods.

In the present study we used the volumetric Karl Fischer titration to estimate these types of water molecules in cyclodextrins by means of the rate of water reaction (related to diffusion from cyclodextrin crystals). "Surface" water molecules are titrated with rates between 1.8–2.8 mM/s for α-cyclodextrin, while for β-cyclodextrin these rates are little bit higher (2.9–3.4 mM/s). The rates corresponding to "strong-bonded" water molecules are approximately tens fold lower (0.05–0.3 mM/s for α-cyclodextrin and 0.15–0.33 mM/s for β-cyclodextrin). The approximate ratio between "surface" and "strong-bonded" water molecules could also be estimated by this simple and rapid method.

Abbreviations
KFT   Karl Fischer titration
aCD   α-cyclodextrin
bCD   β-cyclodextrin

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Introduction

Water is one of the most important compounds in the human life and environment. It is important at planetary and molecular level. Considering the last sentence, the water concentration is one of the main parameter in the bioactive compounds carriers, such as cyclodextrins [1–4]; they are cyclic oligosaccharides with architectures like truncated cones having hydrophobic inner cavities and hydrosolubilizing exterior [5–7]. Bioactive compounds such as drugs, odorant and flavoring compounds, especially those with higher hydrophobicity, can be molecular encapsulated in the cyclodextrin cavity [1, 4, 8–10].

Many factors influence the association–dissociation of the host–guest complex, some of them being related to hydrophobicity [6, 8, 11–13]. In the solid state, water crystallisation molecules exist both in "pure" cyclodextrins and corresponding bioactive compound complexes [4, 14, 15]. In

the complexation process, hydrophobic compounds or moieties are encapsulated in the inner cavities of cyclodextrins by means of van der Waals interactions, and some of water molecules are replaced [3, 11, 12, 15–19]. This aspect can be evaluated by different methods (thermogravimetry, differential scanning calorimetry). In the solid state, cyclodextrins (non-complexed or complexed) contain two types of water molecules: "surface" water molecules (especially those from the crystal surface and some of water molecules which are located between cyclodextrin molecules; the classification of the last type of water molecules as "surface" is hard to do because it depend on the localization of these water molecules in the cyclodextrin crystals—water molecules between cyclodextrin entities, which are situated close to the crystal surface, could be "surface" water, but if these water molecules are located in the "centre" of the crystals, they will be probably "strong-bonded" water molecules; even if they are located in the cyclodextrin intermolecular space) and "strong-bonded" water molecules (especially those from the inner cavity of cyclodextrins) (Fig. 1) [3, 7, 12, 17]. The concentration of these water molecules are relatively difficult to estimate by simple methods.

In the present study we used the volumetric Karl Fischer titration (KFT) to estimate the "surface" water molecules and "strong-bonded" water molecules by means of the rate of water reaction (related to diffusion from cyclodextrin crystals) for natural cyclodextrins (α- and β-cyclodextrin) and to evaluate the ratio between these types of water molecules by using this simple and rapid method.

Materials and methods

Materials

Cyclodextrins used for Karl Fisher water titration and evaluation of "surface" and "strong-bonded" water molecules have analytical purity (≥99 %) and was achieved from Sigma-Aldrich (α-cyclodextrin) and Fluka Chemie AG (β-cyclodextrin); the crystal structure of the commercial cyclodextrins was described previously (α-cyclodextrin have regular prismatic shapes, while β-cyclodextrin have hexagonal shapes, both with a maximum length of 10 μm) [14, 15, 18, 19]. Titrant 5 apura®, solvent apura®, and water standard 1 % apura®, used for two-component Karl Fischer water titration of cyclodextrins, were purchased from Merck&Co., Inc. All alcohols (absolute ethanol, n-propanol, n-butanol) used for solvent hydrophobicity variation in KFT analysis were purchased from Chimopar, Bucharest and were analytical grade. These alcohols were previously dried on molecular sieves (4 Å, Sigma-Aldrich), but the remaining water in alcohols (<0.1 %, according to the KFT analysis) was "neutralized" in the conditioning process.

Karl Fischer water titration

Classical Karl Fischer water titration of α- and β-cyclodextrin were carried out at room temperature by using a Karl Fischer 701 Titrando apparatus from Metrohm (Switzerland); a Metrohm 10 dosing system and 703 Ti

Fig. 1 Schematic representation of "surface" water molecules (especially those from the crystal surface and some of water molecules which are located between cyclodextrin molecules) and "strong-bonded" water molecules (especially those from the inner cavity of cyclodextrins) in natural cyclodextrins and the interaction mechanism with biologically active compounds
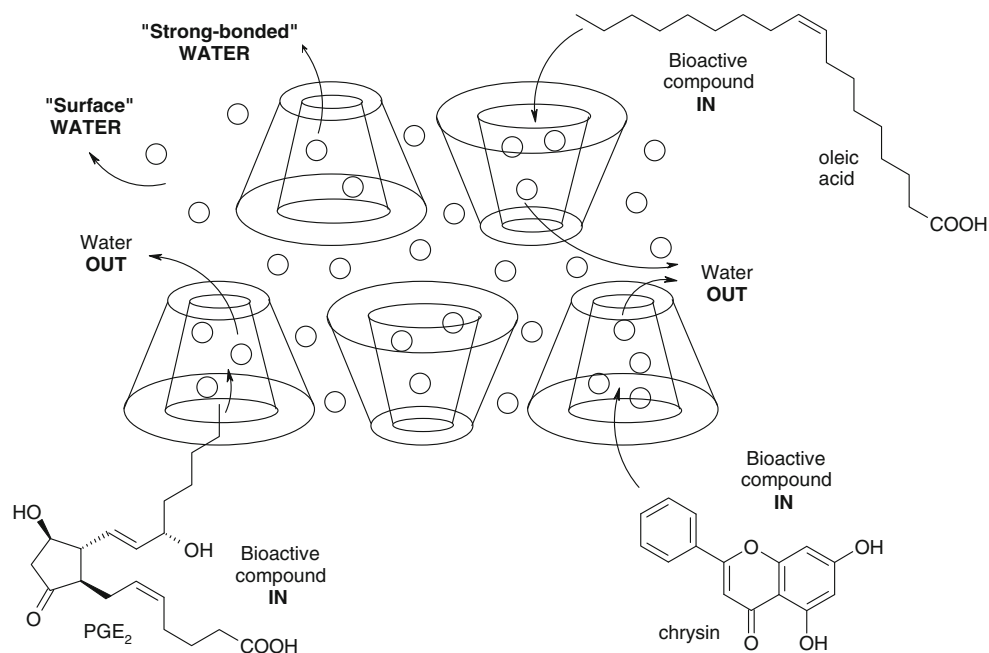
Stand mixing system were also used (both from Metrohm, Switzerland). The two-component technique was used for water determination (Component 1: Titrant 5 apura® and Component 2: Solvent apura®). The titer of component 1 was performed by using Water standard 1 % apura®, standard for volumetric Karl Fisher titration (the titer was 3.7436 mg/g for all samples). The sample amount was ~0.05 g. The method parameters were: $I(pol)$ of 50 μA, end point and dynamics at 250 mV, maximum rate of 5 mL/min, drift was used as stop criterion, with a stop drift of 20 μL/min. The extraction time was 300 s. All determinations were done in triplicate. The hydrophobicity of the solvent was set up for an alcohol (ethanol, propanol, butanol):Solvent apura® ratio of 1:5. The cyclodextrin samples and KFT conditions are presented in Table 1.

Solvent descriptors and statistical analysis

Statistical analysis (analysis of variance, ANOVA) of the experimental data and the correlation analysis (standard multiple regression) between water content and KFT parameters were performed by using the classical statistical parameters: mean, standard deviation for the experimental data, correlation coefficient, F test for the regression equations (an *in house* software was used for basic statistics). Values for the logarithm of the octanol/water partition coefficient (log P) for pure alcohols were collected from [20].

Results and discussion

Karl Fischer water content

Karl Fischer water titration of natural cyclodextrins allow to evaluate only the water molecules from samples by a chemical reaction (Fig. 2), in comparison with other methods such as drying or thermogravimetric methods (where also other volatile compounds could be quantified). In the two-component KFT method, the solvent component contains sulfur dioxide and imidazole, which are dissolved in methanol as imidazolium methyl sulfide (first reaction in Fig. 2). This solvent (without or with other solvents, such as aliphatic alcohols used in this study) is in excess in the KFT reaction vessel and the water titration from samples take place after the "neutralizing" of the remaining water from the solvent (conditioning step). As a result, only the water from the sample studied will react in the iodine redox reaction to give imidazolium methyl sulfate and imidazolium iodide (second reaction in Fig. 2); this reaction is electrically monitored.

The water concentration in α-cyclodextrin samples, determined by KFT in various solvent mixtures, varies in the range of 11.2–12.2 %, but only in the case of methanol–butanol mixture this concentration is higher. The water concentration average is 11.56 ± 0.424 %, but if the last sample is missing, the value is little bit lower (11.36 ± 0.179 %) with an enhanced standard deviation (Table 3). Only in the case of methanol-ethanol solvent system the standard deviation was >1 %, all other standard deviation values being <0.5 %.

The same behavior of KFT process was observed also in the case of β-cyclodextrin samples. The water concentration was in the range of 14.6–16.1 % (Table 2, 3), slightly higher for the methanol–butanol mixture, as in the α-cyclodextrin case; the average of water concentration
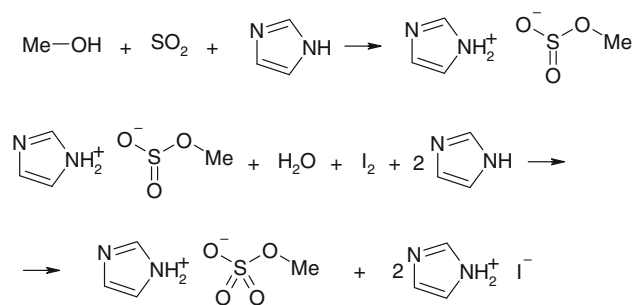
Fig. 2 Karl Fischer reaction (two-component technique, with imidazole)

**Table 1** Cyclodextrin sample codes and conditions used for Karl Fischer water titration

Nr.	Code	Descriere
1	aCD_M	α-Cyclodextrin, KF titration in methanol system
2	aCD_E	α-Cyclodextrin, KF titration in ethanol:methanol system (1:5, v/v)
3	aCD_Pr	α-Cyclodextrin, KF titration in propanol:methanol system (1:5, v/v)
4	aCD_Bu	α-Cyclodextrin, KF titration in butanol:methanol system (1:5, v/v)
5	bCD_M	β-Cyclodextrin, KF titration in methanol system
6	bCD_E	β-Cyclodextrin, KF titration in ethanol:methanol system (1:5, v/v)
7	bCD_Pr	β-Cyclodextrin, KF titration in propanol:methanol system (1:5, v/v)
8	bCD_Bu	β-Cyclodextrin, KF titration in butanol:methanol system (1:5, v/v)

was 15.1 ± 0.22 % ($n = 4$), and 14.71 ± 0.09 % ($n = 3$, without methanol–butanol case). The maximum standard deviation was obtained also for methanol-ethanol mixture.

The water reaction rate differs from one sample to another and even in the same sample the titration behavior has generally three pseudolinear variations, as is revealed by the V/m (mL/g) vs. Time (s) plot for α- and β-cyclodextrin samples (Figs. 3, 4), where V is the titrant volume in the KFT process, and m is the weight of the sample. The volume of the reaction solution is known (60 mL of methanol or ethanol, propanol, butanol/methanol mixture, 1:5 ratio, v/v) and the variation of this volume is insignificant in the titration process (maximum 0.7 and 1 mL of component 1—methanolic iodine solution, with known titer was used for water titration in α- and β-cyclodextrin samples, respectively). The variation of V/m in time allows to evaluate the water reaction rate from the V/m vs. Time slope for all three ranges, because the variation of component 1 volume (with titer of 3.7436 mg/mL) in the KF redox reaction (Fig. 2) allows to calculate the variation of the water concentration (mM) in time from the above mentioned slope (i.e. see Figs. 3 and 4 for the second range).

It can be assumed that the pseudolinear variation of V/m in time is due to the following aspects: the water reaction rate strongly depends on the releasing of water molecules from the cyclodextrin crystals (cyclodextrin have lower solubility in alcohols mixtures), and further from cyclodextrin cavity; the water molecules instantaneously reacts when they are delivered in solution; the overall water

reaction rates are higher in the first range (10–30 s) and it can be assumed that this range corresponding to the "surface" water molecules (which are located on the surface of crystals and between cyclodextrin molecules); in the second range (a pseudolinear variation can be observed also in the approximate range of 30–70 s) the reaction rate is
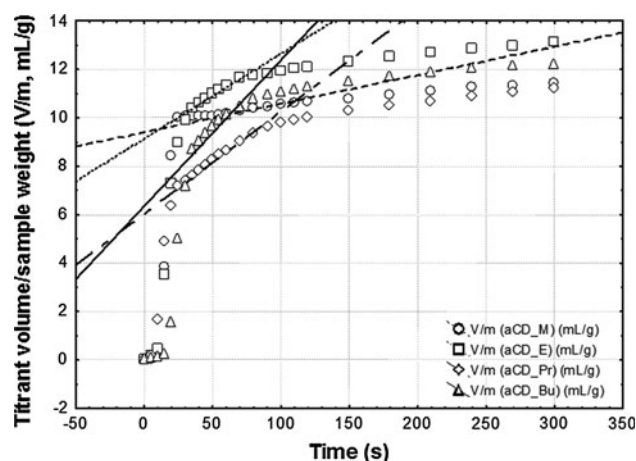
Fig. 3 Variation of titration volume weighted to the sample mass in time for α-cyclodextrin samples
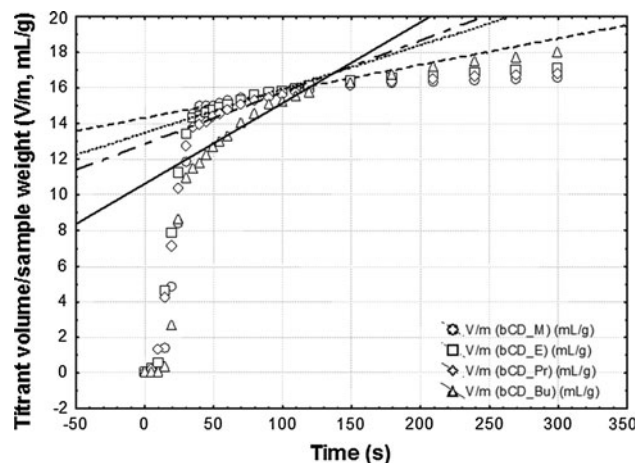
Fig. 4 Variation of titration volume weighted to the sample mass in time for β-cyclodextrin samples

Table 2 log P values for pure alcohols and alcohol mixtures used for Karl Fischer water titration of cyclodextrin samples

No.	Alcohol mixture	log P of pure alcohol	log P solvent mixture
1	Methanol	−0.77 (methanol)	−0.77
2	Methanol:Ethanol (5:1, v/v)	−0.31 (ethanol)	−0.69
3	Methanol:Propanol (5:1, v/v)	0.25 (propanol)	−0.60
4	Methanol:Butanol (5:1, v/v)	0.88 (butanol)	−0.50

Table 3 Karl Fischer water results for α- and β-cyclodextrin samples

No.	Code	Water content[a] (%)	n[b]	No.	Code	Water content[a] (%)	n[b]
1	aCD_M	11.21 ± 0.105	3	1	bCD_M	14.73 ± 0.472	3
2	aCD_E	11.56 ± 1.323	5	2	bCD_E	14.79 ± 0.714	3
3	aCD_Pr	11.32 ± 0.474	3	3	bCD_Pr	14.62 ± 0.654	3
4	aCD_Bu	12.16 ± 0.241	3	4	bCD_Bu	16.12 ± 0.233	2

[a] Water concentration, evaluated by KFT

[b] Number of determinations

lower and it can be assumed that the "strong-bonded" water molecules are titrated in this range; the slope in the third range is lower than the drift considered for finishing the titration process and it corresponds to the titration of the normal humidity from KFT vessel.

Considering these assumptions the "surface" and "strong-bonded" water molecules ratio can be evaluated. Thus, the water reaction rate is higher by an order of magnitude for the first interval, in comparison with the second case (Table 4). The maximum water reaction rate for the first range is obtained for the more hydrophilic solvent (methanol) in both α- and β-cyclodextrin cases (2.7 and 3.4 mM/s, respectively). No dependence exists between this reaction rate and solvent hydrophobicity for the first range. The water reaction rate in the second interval varies from 0.05 to 0.3 mM/s for α-cyclodextrin and from 0.2 to 0.3 mM/s for β-cyclodextrin and seems to be influenced by the hydrophobicity of the solvent mixture (Table 4).

Considering the first two ranges, the ratio between the concentration of "surface" and "strong-bonded" water molecules can be estimated. A ratio of ~ 3 was obtained for the case of α-cyclodextrin samples, but only for more hydrophobic solvent mixtures; this ratio was in the range of 2.7–4 for the case of β-cyclodextrin (Table 4).

Water reaction rate–solvent hydrophobicity correlation

The water reaction rate for the second interval is relatively low due to the fact that it depends on the diffusion of water molecules from inside of the cyclodextrin crystals, most probably from the cyclodextrin cavity; these probably are "strong-bonded" water molecules. This aspect is confirmed by the variation of water reaction rate for the second interval which increases with the solvent mixture hydrophobicity. This could be explained by the possibility of replacing the water molecules from the cyclodextrin cavity ("strong-bonded") by the more hydrophobic alcohols, and further these water molecules are extracted and react with increased rate.

The correlation of water reaction rate with the hydrophobicity of the solvent mixture (log P) conduct to similar equations for both α- and β-cyclodextrin cases (Eqs. 1 and 2); even the number of cases are low ($n = 4$) the correlation is statistically significant, having correlation coefficients of 0.93 and 0.89 and a standard error of estimates lower than 0.05 for both cases.

$$v_{2(\text{aCD})} = 0.715(\pm 0.143) + 0.817(\pm 0.221) \times \log P_{\text{solv.mix}}$$
$$n = 4, \ r = 0.934, \ s = 0.046, \ F = 14$$
$$(1)$$

$$v_{2(\text{bCD})} = 0.630(\pm 0.149) + 0.619(\pm 0.231) \times \log P_{\text{solv.mix}}$$
$$n = 4, \ r = 0.885, \ s = 0.047, \ F = 8$$
$$(2)$$

Conclusion

The following conclusions can be drawn among the Karl Fischer water titration in natural cyclodextrins: (1) KFT is an appropriate method to evaluate the total water concentration in cyclodextrin samples, but the hydrophobicity of solvent used is important; a more hydrophobic solvent such as butanol can conduct to a relatively higher water concentration (as results from KFT calculus) and it must be necessary to reconsider the KF parameters for a correct evaluation (i.e. the drift); (2) the water reaction rate from KFT process could be a good indicator on the concentration of "surface" and "strong-bonded" water molecules from natural cyclodextrins; (3) the "strong-bonded" water molecules are especially those from the cyclodextrin cavity, this affirmation being sustained by the KFT behavior: the hydrophobicity of the solvent is very important because it could replace the water molecules from cyclodextrin cavity and favors the diffusion/extraction of water in KFT process. Finally, the Karl Fischer water titration is a rapid and efficient method for evaluation of water molecule types from cyclodextrins.

Table 4 Water reaction rates and the ratio between "surface" and "strong-bonded" water molecules from Karl Fischer titration for three significant time ranges (10–30 s, 30–70 s, and 70–300 s)

No.	Code	v_1 (mM/s)	v_2 (mM/s)	v_3 (mM/s)	$c_{sf.w}/c_{sb.w}^{a}$
1	aCD_M	2.73 ± 0.11	0.05 ± 0.02	0.02 ± 0.005	–
2	aCD_E	2.51 ± 0.22	0.19 ± 0.09	0.04 ± 0.010	–
3	aCD_Pr	1.82 ± 0.32	0.24 ± 0.02	0.05 ± 0.005	3.03 ± 0.28
4	aCD_Bu	2.40 ± 0.26	0.29 ± 0.04	0.04 ± 0.003	2.88 ± 0.78
5	bCD_M	3.39 ± 0.39	0.19 ± 0.04	0.02 ± 0.009	3.86 ± 2.45
6	bCD_E	3.02 ± 0.81	0.15 ± 0.03	0.04 ± 0.007	3.98 ± 2.63
7	bCD_Pr	2.92 ± 0.70	0.16 ± 0.04	0.03 ± 0.005	3.26 ± 2.03
8	bCD_Bu	3.29 ± 0.05	0.33 ± 0.03	0.08 ± 0.002	2.67 ± 0.24

[a] The ratio between concentrations of "surface" and "strong-bonded" water molecules

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