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Solubility Enhancement of Low Soluble Biologically Active Compounds by β-Cyclodextrin and Dimethyl-β-cyclodextrin

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Abstract

The solubility enhancement of triflumizole by complexation with β-cyclodextrin and with dimethyl-β-cyclodextrin is compared with respect to the different physico-chemical properties of the host molecules. Although the inclusion reaction constants are rather similar for both complexation reactions, a completely different temperature dependence of the host-guest interaction is observed, which indicates a change of the reaction mechanisms. Moreover, the influence of ethanol as cosolvent is studied.

Introduction

Cyclodextrins are essential ingredients in pharmaceutical technology for the solubility enhancement of drugs [1–3]. They play also an important role for the increase of the bioavailability of other biologically active compounds like biozides [4]. The most pronounced property of cyclodextrins is the possibility to form inclusion complexes with small or even mediumsized organic compounds, which changes the physico-chemical behavior of the guest molecules, like the reactivity or the solubility [5–7]. Such a solubility enhancement depends on the affinity between the molecules to the interior of the cyclodextrin as host molecules. The binding affinity between the cyclodextrin molecule and the inclusion compound, quantitatively described by the complexation constant K, is influenced by the molecular properties of the guest molecule as well as of the cyclodextrin used. Steric as well as electronic parameters influence the inclusion complexation as the molecular surface of the guest molecule should fit as accurate as possible into the interior of the cyclodextrin. Moreover, the electrostatic potential and the hydrophobicity affect the binding affinity to a high extent. The flexibility of the host molecule is one additional parameter, which is responsible for the geometry and consequently for the stability of the complex. At least also these parameters, which are generally important for chemical equilibria, like the temperature or the solvent have to be taken into account for considerations of cyclodextrin inclusion interactions.

The properties of natural cyclodextrins, in particular the size can be varied by the number of glucose units connected in the macromolecule. Prediction models for the association of some organic compounds have been developed with the help of linear as well as nonlinear QSAR techniques [8–10], which show that the inclusion mechanism is not the same for different cyclodextrins, as the flexibility of the cyclodextrins varies with the ability to build up hydrogen bonds in the interior.

Beyond the natural cyclodextrins also some derivatives have been synthesized with modifications at the hydroxyl groups of the rim. E.g. the methylation of native β-cyclodextrin (CD) has been performed by phase-transfer-catalysis [11]. The product is a mixture of methylated CD (randomly methylated CD), with 60-70% heptakis(2,6-di-O-methyl)-β-cyclodextrin (DMCD), 10-15% heptakis(2,3,6-tri-O-methyl)-β-cyclodextrin and some monomethylated derivatives. In the following Table 1 some physico-chemical parameters are given for CD and DMCD [5].

Both compounds show quite different solubilities in water and contrary temperature coefficients. The water solubility of DMCD at 25 °C is significantly higher than that of CD but sparingly soluble at higher temperatures. A possible explanation for this phenomenon is, that strong intramolecular hydrogen bonds exist in the interior of CD [12, 13].

As CD and DMCD possess different physico-chemical properties, also their ability to enhance the solubility of various biological active compounds by inclusion complexation could be different. This has been investigated in detail on the complexation with triflumizole (TF), a systemic fungizide. The association constants between TF and CD as well as DMCD have been determined in dependence on the temperature and on different concentrations of ethanol as cosolvent in order to get further insight information about the solubility enhancement of cyclodextrins.

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Table 1. Physico-chemical properties of CD and DMCD

	CD	DMCD
Molecular formula	$C_{42}H_{70}O_{35}$	$C_{56}H_{98}O_{35}$
Molecular weight	1134 g	1330 g
Melting point	280 °C	295–300 °C
Internal diameter	0.78 nm	0.5 nm
External diameter	1.53 nm	
pKa (25 °C)	12.3 potentiometr.	
Solubility in water	1.88 g/100 ml (25 °C)	>80 g/100 ml (25 °C)
	3.49 g/100 ml (40 °C)	
	positive solubility coeff.	negative solubility coeff.

Experimental

Reagents

Triflumizole (TF), (E)-4-chloro-α,α,α-trifluoro-N-(1-imidazol-1-yl-2-propoxy-ethylidene)-o-toluidine (IUPAC), (E)-1-[1-[[4-chloro-2-(trifluoromethyl)phenyl]-imino]-2-propoxyethyl]-1*H*-imidazole (C.A.), CAS Nr. *99 387-89-0*, was provided by Nippon Soda Co. Ltd. (Japan) with a purity of >99%. CD was purchased from Roquette Frères (Lestrem, France) as Kleptose® with a humidity of 14% (w/w). DMCD was obtained from Ringdex (Syntapharm Ref. Nr: 1712) Ethanol was of analytical reagent grade; the water used in this study was bidistilled.

Solubility studies

Solubility measurements and the determination of the saturation concentrations were carried out adding excess amounts of TF (concentration range 4×10^{-4} to 6×10^{-3} mol L^{-1}) to water, water/cosolvent mixtures and CD or DMCD solutions, respectively. After stirring the samples in a temperature controlled water bath until equilibrium was reached (generally 24 hours, and 36 hours for the complexation with CD and DMCD), the concentrations of dissolved TF were determined by electron absorption spectroscopy using a Perkin Elmer UV/VIS Spectrometer Lambda 16 (Perkin Elmer, Norwalk, CT, USA) at a wavelength of 295nm. The saturation concentrations were estimated at different temperatures (25 °, 30 °, 34 °, 38 °C). The temperatures were kept constant ±0,5 °C. Six measurements were taken at each temperature and the average value was used.

Solutions with ethanol were prepared at concentrations of 4, 8 and 12% (w/w). Stock solutions of CD and DMCD were prepared and used for the solubility measurements as well as for the determination of the equilibrium constants. Due to the instability of the solutions, the spectra were recorded immediately after dilution.

The overall complexation constants K were estimated by the solubility method, assuming an one step equilibrium, varying the concentration the cyclodextrins from 0–6 $\times 10^{-3}$ mol L^{-1}, according to the method of Higuchi and Connors [14].

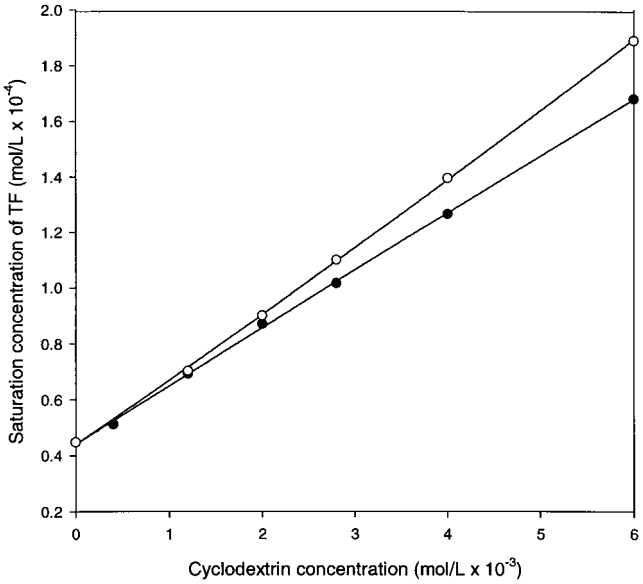

Figure 1. Saturation concentration of dissolved TF at increasing concentrations of CD (solid circles) and DMCD (open circles) at 25 °C.

Table 2. Saturation concentration and overall equilibrium constant K of TF/cyclodextrin inclusion complexes at 25 °C and a concentration of the cyclodextrins of 6×10^{-3} mol L^{-1}.

	Sat. conc. (10^{-4} mol L^{-1})	K (L mol^{-1})
TF in water	0.448	
TF in CD/water	1.681	475.6
TF in DMCD/water	1.927	623.5

Results

The solubility enhancement of TF in water in dependence on the temperature and various cosolvents of different concentrations has been studied extensively already [4, 15]. In the present investigation the solubility enhancement caused by complexation with CD in comparison to that with DMCD has been determined at different temperatures as well as under the influence of small amounts of ethanol as cosolvent.

The solubility enhancement of TF at 25 °C in dependence on the concentration of CD and DMCD is given in Figure 1.

The solubility of TF increases with raising amounts of both CDs, due to the increasing concentration of TF in the complexed form in the thermodynamic equilibrium. DMCD dissolves TF slightly better, as the slope of the plot is more steep. The resultant saturation concentrations of TF and the overall equilibrium constants of the inclusion reaction are given in Table 2.

CD at a concentration of 6×10^{-3} mol L^{-1} dissolves the 3.75fold amount of TF in comparison with pure water, the increase of the saturation concentration of TF due to complexation with DMCD is 4.3 fold.

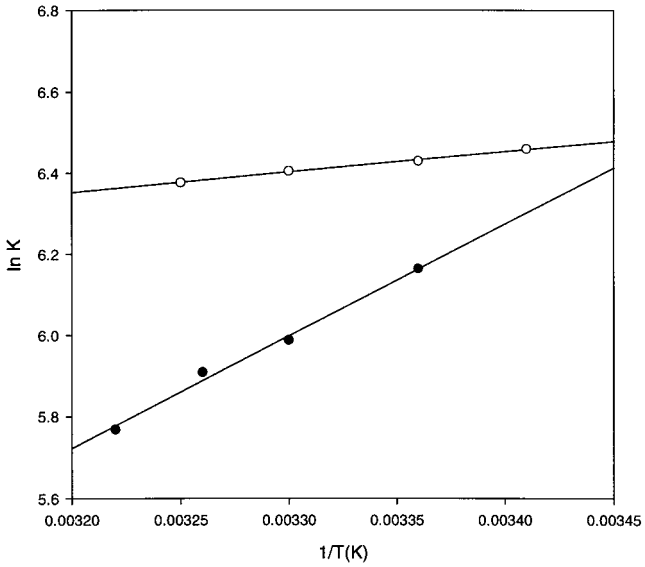

Figure 2. Van't Hoff plot of the logarithm of the overall equilibrium constant K of the inclusion complex of TF in the presence of CD (solid circles) and DMCD (open circles) at a cyclodextrin concentration of 6×10^{-3} mol L^{-1}.

Table 3. Thermodynamic parameters of the overall complexation reaction between CD and TF as well as between DMCD and TF.

	K(L mol^{-1})	ΔG(kJ/mol)	ΔH(kJ/mol)	ΔS(JK/mol)
CD	475.6	−15.3	−23.0	−25.9
DMCD	623.5	−16.0	−4.2	+39.4

Temperature dependence

Although the shape of the rims of CD and DMCD show remarkable different structures, the affinities of the cyclodextrins to TF are similar to some extent, as the equilibrium constants are in same order of magnitude. In contrary to that the temperature dependence of the solubilities and consequently the thermodynamic parameters of the association reactions are completely different. The influence of the temperature on the overall equilibrium constant K is demonstrated in Figure 2.

According to the Van't Hoff equation the plot of the logarithm of the association constants in dependence on the reciprocal temperature results in straight lines, but significantly different for both cyclodextrins under consideration. Whereas CD shows a pronounced temperature dependence, the inclusion constant of DMCD increases much less with raising temperature. The estimated thermodynamic parameters for both inclusion reactions are given in Table 3.

As a consequence of the slopes in the Van't Hoff plot (Figure 2) the reaction enthalpies for both inclusion reactions are remarkably different. From the overall free energy of the reaction, calculated from the equilibrium constant and the measured reaction enthalpies the reaction entropies can be calculated. For the reaction of CD with TF a negative value for the reaction entropy is observed, whereas the corresponding value for the DMCD inclusion reaction appears to be positive.

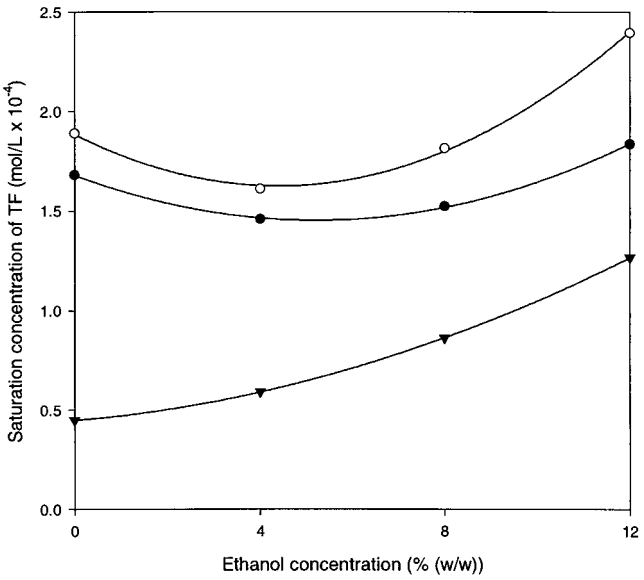

Figure 3. Saturation concentration of dissolved TF in water (solid triangles), at 6×10^{-3} mol L^{-1} of CD (solid circles) and DMCD (open circles) in dependence of various ethanol concentration at 25 °C.

Influence of ethanol as cosolvent

Both, the solubility of low soluble compounds and their association equilibria with cyclodextrins are strongly influenced by the solvent [16–18]. Therefore, the addition of small amounts of cosolvents only changes the solubilites of biologically compounds to an high extent. The influence of various cosolvents on the solubility of TF and on the inclusion reaction with CD has been studied in detail already [15]. In Figure 3 the influence of the concentration of ethanol on the solubility of TF in pure water is compared to that in the presence of 6×10^{-3} mol L^{-1} CD (solid circles) and DMCD (open circles).

The saturation concentration of TF in water ethanol mixtures increases with raising cosolvent concentrations (a factor of 2,83 is observed for 12% (w/w) ethanol). For the association complexes between CD or DMCD with TF a decrease of dissolved TF can be recognized at low cosolvent concentration followed by an increase at higher ethanol concentrations. For both cyclodextrins the addition of ethanol leads to a destabilization of the inclusion complexes, probably caused by the competition of the inclusion reaction of TF and the cosolvent molecules. At higher ethanol concentration the diminished host guest affinity is compensated by the better solvation of TF in the solvent mixtures. The destabilization of the complex with CD is stronger than with DMCD, which is demonstrated in Figure 4.

Consequently, the solubility of TF in presence of DMCD at higher cosolvent concentration is essentially larger. The overall, experimentally feasible solubility of TF is defined as the sum of the dissolved complexed compound and the dissolved free TF, which is in thermodynamic equilibrium with the complexed species. The ratio between the two forms depends on the complexation of TF and the host molecule, whereas the concentrations of the different species depend on the solubility of the free and the complexed form. The

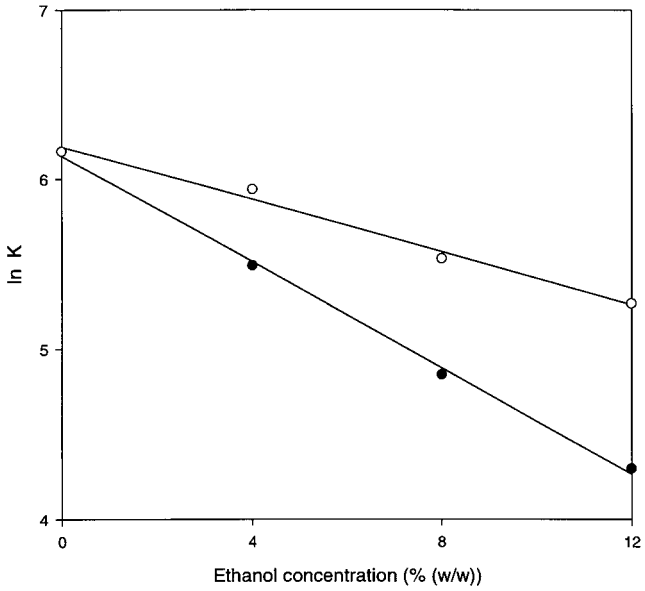

Figure 4. Logarithm of the overall equilibrium constant K of the inclusion complexation of TF in the presence of CD (solid circles) and DMCD (open circles) at a cyclodextrin concentration of 6×10^{-3} mol L^{-1} in dependence of various ethanol concentrations at 25 °C.

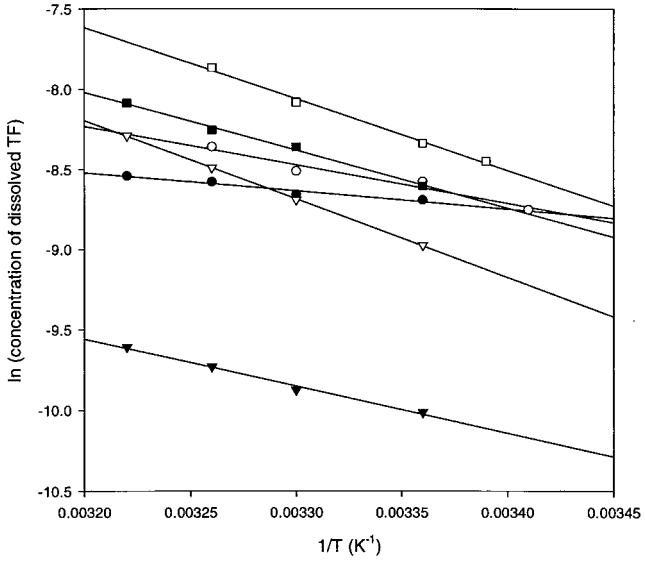

Figure 5. Van't Hoff plot of the logarithm of the saturation concentration of dissolved TF in water (solid triangles) and 12% (w/w) ethanol (open triangles), at a CD concentration of 6×10^{-3} mol L^{-1} (solid circles) and with 12% (w/w) ethanol (solid squares) and at a DMCD concentration of 6×10^{-3} mol L^{-1} (open circles) and with 12% (w/w) ethanol (open squares).

influence of the temperature on the various solublities results from the temperature dependence of the association reaction and from both contributions of the solubilities of free and complexed molecules. In Figure 5 the Van't Hoff plots of TF in water and in water-ethanol mixture is compared with solutions of 6×10^{-3} mol L^{-1} CD or DMCD.

The solubility of TF in pure water increases significantly with raising temperature. The corresponding dependence of the much higher solubility in 12% (w/w) ethanol shows a stronger temperature dependence. In contrary, the temperature dependence of the solubilities in presence of CD is much smaller, a little bit larger for DMCD. Addition of 12% (w/w) ethanol increases the slope of the temperature dependence for both host molecules. Also in this case the slope of the dependence of DMCD is higher and is very similar to the dependence of the solubility of TF in absence of cyclodextrins (open triangles).

Discussion

Molecular calculations on TF/CD inclusion complexes have shown, that one side chain of the molecule is included into the interior of the host, whereas the residual is associated at the outer surface of the macromolecule and a part of the molecule is exposed to the solvent [4]. As a consequence of this study also the properties of the complete molecular surface of cyclodextrins as well as interactions of the solute with the solvent molecules have to be taken into account for a discussion of the complexation reaction. Moreover, only the overall reaction, which results from the experimentally observed concentrations of TF in dependence on cyclodextrin concentrations, can be considered. Thus, the experiments give some information about the thermodynamic parameters of the overall reaction only. Evidently a series of elementary reaction steps are included in this overall reaction, which cannot be split up without further experimental evidences. Therefore the experiments give some information about the thermodynamic parameter of the overall reaction only. Inclusion reactions of the cyclodextrins with TF are competing with association with water or cosolvent molecules, leading to reaction steps with individual reaction enthalpies and entropies. Nevertheless, from the overall reaction parameters it can be concluded, that the thermodynamic parameters for the association reaction of CD and DMCD are significantly different, although their overall complexation constants are similar. CD shows enthalpy-entropy compensation, which is observed very often for cyclodextrins inclusion reactions [19–21], whereas the complexation between DMCD and TF is mainly entropically driven, as the reaction enthalpy is surprisingly low and the resulting overall reaction entropy occurs to be larger and moreover positive.

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