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Solubility Enhancement of Triflumizole by Host–Guest Interaction with β-Cyclodextrin

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Summary. The solubility enhancement of triflumizole, a systemic fungicide, by β-cyclodextrin inclusion complexation was investigated by electron absorption spectroscopy. The respective association constant determined by different methods was estimated to $470 \pm 20\,M^{-1}$ in aqueous solution. A model for the host–guest complexation was deduced by molecular calculations.

Keywords. β-cyclodextrin; host–guest complex; inclusion complex; molecular modeling; solubility enhancement; triflumizole.

Löslichkeitsverbesserung von Triflumizole durch *Host–Guest*-Complexierung mit β-Cyclodextrin

Zusammenfassung. Die Löslichkeitsverbesserung des systemischen Fungizids Triflumizole durch Komplexierung mit β-Cyclodextrin wurde mittels Elektronenabsorptionsspektroskopie untersucht. Die entsprechende Stabilitätskonstante wurde durch verschiedene Methoden erfaßt und betrug im wäßrigen Medium $470 \pm 20\,M^{-1}$. Ein Modell für den *Host–Guest*-Complex wurde durch Molekülrechnungen erstellt.

Introduction

Enhanced solubility in water and the consequently improved activity and bioavailability is not only important for slightly soluble drugs but also for pesticides, *e.g.* fungicides. A possible method providing solubility enhancement seems to be the inclusion of the substances by convenient molecules like cyclodextrins. Cyclodextrins belong to a class of cyclic compounds built up from α-*D*-glucose subunits forming a cone. A wide variety of organic molecules can be complexed into the interior of these compounds [1]. The number of the glucopyranose units define the size of the cavity; in β-cyclodextrin, 7 units are connected. The inclusion of molecules and the spatial restrictions caused by the cavity influence the reactivity, the spectroscopic properties of the guest molecules and also their physicochemical behaviour, like for example, the solubility. Some association complexes of various cyclodextrins with organic molecules or drugs were investigated extensivily [2–5]; studies on the solubility enhancement of drugs were also reported [6, 7]. It is evident that the change of the microenvironment from the hydration shell in aqueous solution to the more hydrophobic interior of the host molecules is responsible for the

modification of the molecular properties. The aim of the present study was to investigate the solubility enhancement of triflumizole, a systemic fungicide, caused by β-cyclodextrin. Therefore solubility studies were performed in aqueous medium as well as in mixtures of ethanol/water and dimethyl sulfoxide/water to examine the influences of solvent polarity and electrostatic interactions on the spectroscopic properties of the guest molecules in comparison to those of the triflumizole and β-cyclodextrin associates. Stability constants for the complex were determined using different methods. Furthermore, triflumizole/β-cyclodextrin complexes in the solid state were prepared and characterized by different spectroscopic methods.

To evaluate the possibilities of the association and to give some information on the structure of the complex, a model of the host–guest complex between triflumizole and β-cyclodextrin was generated by molecular modeling techniques.

Experimental

Materials

Triblumizole was obtained from Nippon Soda Co. Ltd. (Japan) with a purity of >99%. β-cyclodextrin (*β-CD*) was provided by Roquette Frères (Lestrem, France) as Kleptose® with a humidity of 14% (w/w). Ethanol, dimethyl sulfoxide (*DMSO*), dioxane and trichloromethane were of analytical reagent grade, water used in this study was bidistilled.

Preparation of solid triflumizole/β-CD complexes

To achieve triflumizole/*β-CD* complexes in the molar ratio 1:2 in the solid state, an appropriate amount of triflumizole was dissolved in trichloromethane to obtain a concentration of 40% (w/w). The solution was added under stirring to a pasty slurry of *β-CD* in water and kneaded to a homogeneous paste. Kneading was continued until the solvent was evaporated. The resulting product was dried under vacuum over night, then grinded to a powder and dried again till weight constancy [8].

Physical mixtures were prepared by grinding pure triflumizole and *β-CD* in a molar ratio of 1:2 in a mortar [9].

Electron absorption spectra

The absorption spectra were recorded on a Hitachi U3501 spectrophotometer at 25 ± 1 °C. Samples were prepared from saturated solutions of triflumizole by 1:1 dilution. *β-CD* was added as solid. Due to the instability of the measuring solutions the spectra were recorded immediately after dilution. The concentration of the stock solution was checked spectrophotometrically. Spectra from dioxane solutions were obtained by dilution of a 10^{-3} *M* stock solution. For the measuring solutions, solvents for spectroscopy were used (Merck).

Thermal analysis was performed on a Perkin Elmer DSC 7 (Perkin Elmer, Norwalk, CT, USA) at a heating rate of 10 K·min^{-1} in the range of 300 to 400 K. Sample weights varied from 3 to 6 mg.

Determination of stability constants

The stability constants of the inclusion compound in solution were estimated by different methods.

The classical method for measurements of equilibrium constant is based on the equations and plots of *Benesi–Hildebrand* [10] and *Scott* [11],

$$c_B^0\left(\frac{1}{\alpha}\right) = c_A^0 + \frac{1}{K}\left(\frac{1}{1-\alpha}\right),$$

where c_A^0 and c_B^0 are the initial concentrations, K stands for the association constant, and α is defined by

$$\alpha = \frac{|E - E_\infty|}{|E_\infty - E_0|}.$$

E_0 and E_∞ are the extinction values of the pure chromophore and the association complex, respectively, whereas E is the actual extinction value of the chemical equilibrium. It has been shown, that the accuracy of the results obtained by such a procedure depends on the concentration scale [12, 13]; besides, the absorption spectra of the association complexes have to be known.

The concentration range of triflumizole is very limited in aqueous solution due to its very poor solubility. Additionally the extinction value of the pure complex is not very easy to obtain, due to the relative small equilibrium constant of the complex.

Another method used in this study, which prevents the mentioned restrictions, has been developed recently [3]. In this method the association constant (K) is determined by an iterative procedure. Experimental (E_{exp}) and calculated extinction (E_{calc}) are fitted together at various wavelengths:

$$E_{calc} = \varepsilon_{CD} \cdot c_{CD} + \varepsilon_{TF} \cdot c_{TF} + \varepsilon_{IC} \cdot c_{IC}$$

$$\sum_c (E_{exp} - E_{calc})^2 \Rightarrow \text{minimum}$$

c_{CD}, c_{TF}, and c_{IC} are the equilibrium concentrations of *β-CD*, triflumizole and the inclusion complex, respectively and ε_{CD}, ε_{TF} and ε_{IC} are the corresponding molecular extinction coefficients.

According to this method, solutions were prepared diluting a saturated aqueous solution of triflumizole 1:1, adding various amounts of solid β-cyclodextrin (between $1 \cdot 10^{-4}$ and $1 \cdot 10^{-2}$ *M*). The extinction data were collected and evaluated for 235 nm and 295 nm on a personal computer.

Another method to determine the stability constant is based on monitoring changes in solubility of the substrate by addition of complexing agent. The saturation concentration (G) of the guest substance and its changes in solubility are plotted as a function of cyclodextrin concentration. Thus, a phase-solubility diagram is obtained. The apparent complex stability constant (K) can be calculated from the straight line portion of this diagram according to the equation of *Higuchi* and *Connors* [14, 15]:

$$K = \frac{\operatorname{tg}\alpha}{G \cdot (1 - \operatorname{tg}\alpha)}; \qquad \operatorname{tg}\alpha \frac{G_T - G}{CD_T}.$$

G is the saturation concentration of triflumizole, G_T and CD_T are the apparent solubilities of triflumizole and *CD*, resp., and α is the angle of the slope of the diagram.

The measurements were carried out by suspending excess amounts of triflumizole in solutions of *β-CD* in the concentration range of $4 \cdot 10^{-4}$ to $6 \cdot 10^{-3}$ *M*. After stirring the samples at 25 ± 1 °C until equilibrium was reached (36 h), the concentration of dissolved triflumizole was determined by electron absorption spectroscopy using a Perkin Elmer UV/VIS Spectrometer Lambda 16 (Perkin Elmer, Norwalk, CT, USA) at a wavelength of 295 nm.

To investigate the influence of solvents on the complexation behaviour of the compounds, solubility studies were carried out with pure water as well as mixtures of water/ethanol and water/*DMSO*. Solubility measurements and phase-solubility diagrams were elaborated for pure water and for each of the different mixtures of solvents.

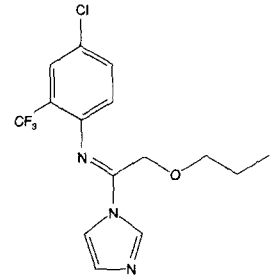
Molecular modeling on the inclusion complexes

Molecular modeling was performed on the host–guest complex between triflumizole and β-cyclodextrins, using the MM3 force field of *Allinger* [16] on an IBM RISC 6000/375 work station.

Starting from different positions of triflumizole in respect to *CD*, the geometries of the association complexes were minimized.

Results and Discussion

The systemic fungizide triflumizole is an imidazol derivative with the following characteristics:



Triflumizole [17, 18]: (*E*)-4-chloro-α,α,α-trifluoro-N-(1-imidazol-1-yl-2-propoxyethylidene)-*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; $C_{15}H_{19}ClF_3N_3O$, MG = 345.7.

Triflumizole forms colourless crystals, m.p. 63.5 °C; v.p. 0.0014 mPa (25 °C); solubility (20 °C): 12.5 mg/l (water), 2.22 kg/l (trichloromethane), 17.6 g/l (hexane), 639 g/l (xylene); K_{OW}: 25; basic pK_a: 3.7 (25 °C).

The electron absorption of triflumizole is given in Fig. 1, together with the spectrum of the inclusion complex with β-cyclodextrin. A comparison of the spectral data is given in Table 1.

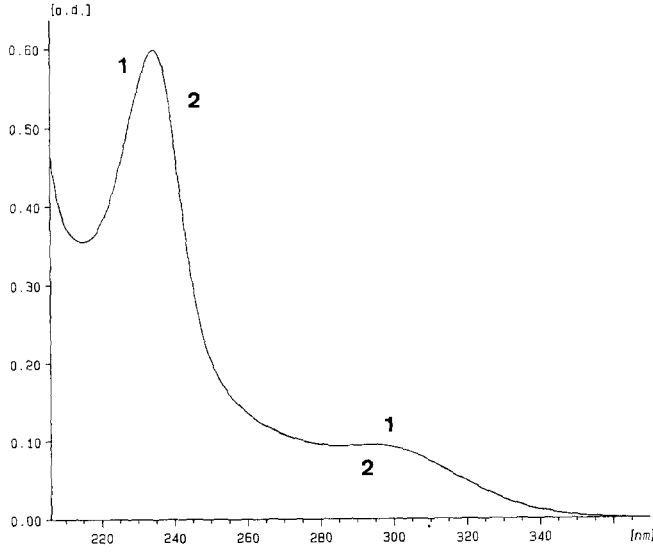


Fig. 1. Electron absorption spectra of pure triflumizole (1) and of the triflumizole/cyclodextrin inclusion complex (2) in aqueous solution

Table 1. Spectral data of triflumizole in different environments (wavelengths in nm, molar extinction coefficient in $mol \cdot l^{-1} \cdot cm^{-1}$)

	λ_{max}	ε
Triflumizole	234.5	25800
	293.8	4050
Triflumizole/*β-CD* inclusion	236.2	25400
complex in water	shoulder	3990 ($\lambda = 293.8$)
Triflumizole in dioxane	240.5	25300
	301	5200

The spectra are characterized by two absorption bands (Fig. 1, Table 1). The maximum were at $\lambda_{max} = 293.8$ nm and $\lambda_{max} = 234.5$ nm for the free triflumizole with extinction coefficients of $\varepsilon = 4050$, and $\varepsilon = 25800$, respectively. Association with β-cyclodextrin shifts the shorter wavelengths bathochromically ($\lambda_{max} = 236.2$ nm) and decreases the extinction coefficient of the chromophore slightly ($\varepsilon = 25400$). The intensity of the absorption band at higher wavelength decreases, too. No absorption maxima, but a pronounced shoulder can be observed in the spectrum. The corresponding extinction value at the peak wavelength of free triflumizole was estimated as $\varepsilon = 3990$.

The shift of the absorption spectrum of triflumizole is evidently caused by the change of the environment, as the spectral data in dioxane – a more apolar, aprotic solvent – show: $\lambda_{max} = 301.0$ nm ($\varepsilon = 5200$) and $\lambda_{max} = 240.5$ nm ($\varepsilon = 25300$). The bathochromic shift is increased again, because the interaction of the solvation shell with dioxane as solvent is stronger than in the interior of cyclodextrin, which does not cover the guest molecule completely.

Equilibrium constant of the host-guest complex in aqueous solution

In Table 2, a comparison is given between the inclusion complex equilibrium constant obtained by concentration dependence measurements and solubility experiments. A sufficient good agreement can be recognized, but the accuracy of the latter is much better, due to the experimental difficulties of the concentration dependence method applied on the less soluble compound.

The complex constant of about $470\ M^{-1}$ can be estimated with both methods, in agreement with a 1:1 complexation. The accuracy of the value is much better for the solubility method than for the iteration method, for experimental reasons.

Table 2. Equilibrium constant of the inclusion complex of triflumizole and β-cyclodextrin

Method	$K[M^{-1}]$	Error range
Iteration method	460	±30
Solubility method	470	±20

Table 3. Solubility of triflumizole and association constant (K) for different mixtures of water-ethanol and water-*DMSO*

Solvent	Conc. [%(v/v)]	G[mol·l^{-1}]	tgα	K[M^{-1}]
Ethanol	0	$0.42 \cdot 10^{-4}$	0.019	469
	5	$0.61 \cdot 10^{-4}$	0.014	226
	10	$0.82 \cdot 10^{-4}$	0.012	141
	15	$1.22 \cdot 10^{-4}$	0.011	101
DMOS	1	$0.46.10^{-4}$	0.015	339
	2	$0.49 \cdot 10^{-4}$	0.013	264
	4	$0.56 \cdot 10^{-4}$	0.011	189
	10	$0.81 \cdot 10^{-4}$	0.007	86

This equilibrium constant indicates a moderate complex stability with a sufficient amount of complexed species in aqueous solution. On dilution, the free compound is immediately available, which is important for the biological activity of the substance.

Inclusion complexation in aqueous solutions partially containing ethanol resp. dimethyl sulfoxide

As the stability of the inclusion complex between triflumizole and β-cyclodextrin is mainly controlled by the competition of the interactions between solute and solvent on one side and solute and complexing molecule on the other side, the equilibrium constant is highly solvent dependent. Increasing solvent interaction of more hydrophobic solvents leads to increasing solubility but destabilizes the association complex. This is demonstrated in Table 3, where the solubility of triflumizole and the host–guest complexation constant are compared for different mixtures of water-ethanol and water-*DMSO*.

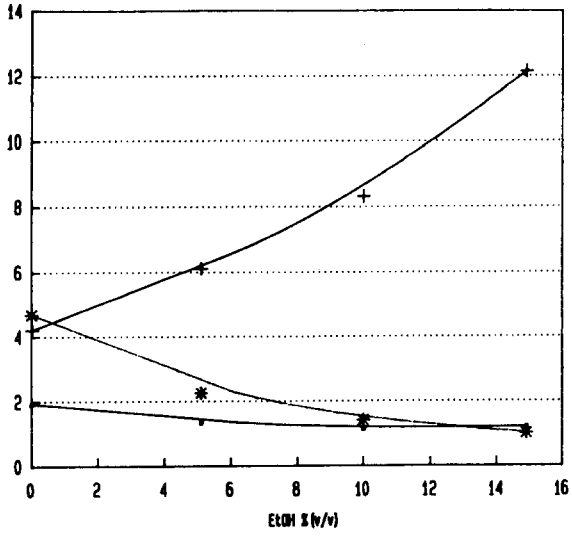


Fig. 2. Dependence of the solubility of triflumizole, the equilibrium constant of the association complex, and tgα on the concentration of ethanol in water, —·— tg$\alpha \cdot 10^{-2}$; —×— G[mol·l^{-1}]·10^{-5}; —*— K[M^{-1}]·10^{2}

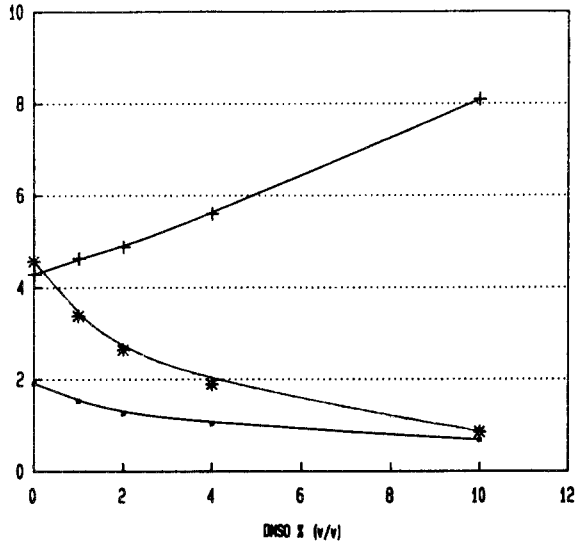


Fig. 3. Dependence of the solubility of triflumizole, the equilibrium constant of the association complex, and tgα on the concentration of *DMSO* in water, —·— tg$\alpha \cdot 10^{-2}$; —×— $G\,[\mathrm{mol \cdot l^{-1}}] \cdot 10^{-5}$; —*— $K\,[M^{-1}] \cdot 10^{2}$

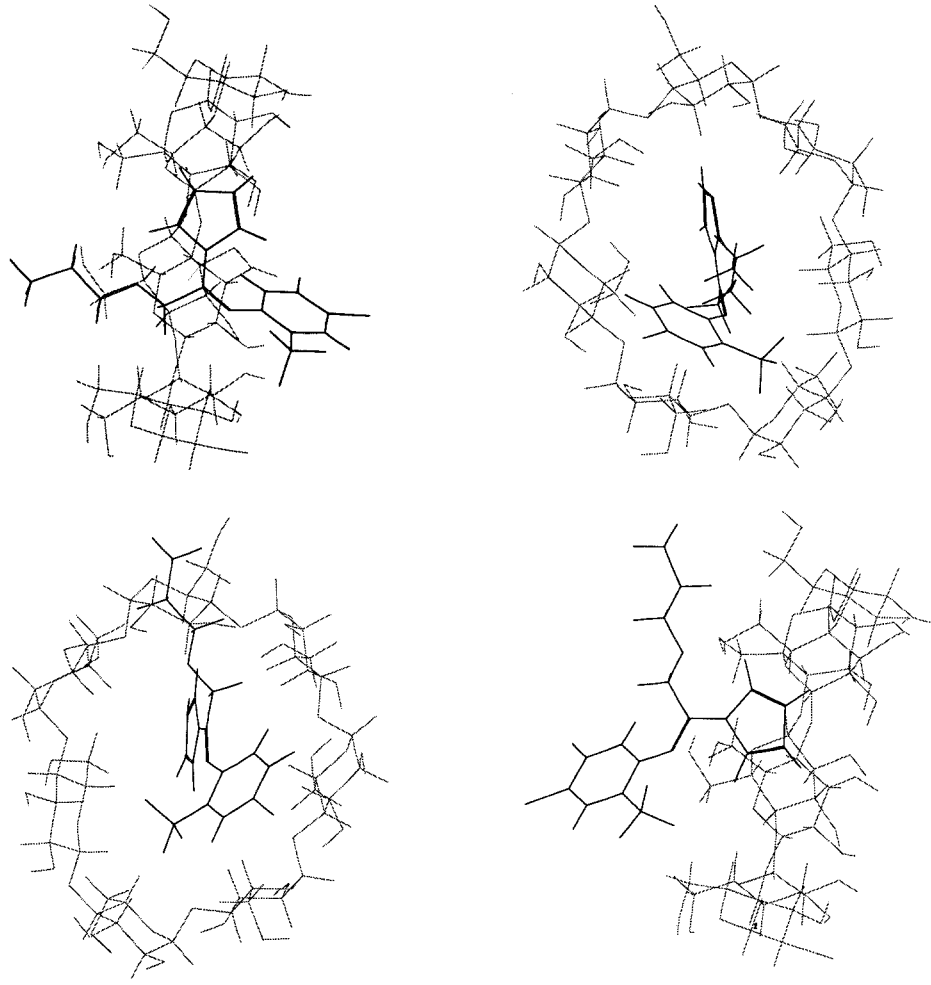


Fig. 4. Minimized geometries of the association complex of triflumizole with cyclodextrin

As expected, the saturation concentration (G) of triflumizole increased with rising amounts of ethanol resp. *DMSO* in the solvent. The solubility of triflumizole was enhanced linearly by addition of *β-CD*. The solubility improving effect of *β-CD*, however, was diminished in higher ethanol or *DMSO* concentrations, which is expressed in the tgα values of the solubility diagrams.

As no deviation from linearity is noted, all phase-solubility diagrams can be classified as A_L-type-diagrams. The apparent stability constants (K) as a measure of inclusion complexation were calculated according to *Higuchi* and *Connors* [14], assuming a 1:1 complex formation in solution.

In Fig. 2 (ethanol/water mixtures) as well as in Fig. 3 (*DMSO*/water mixtures) the increasing solubility with corresponding decreasing equilibrium constant is demonstrated. The influence of the aprotic solvent *DMSO* is stronger than that of the protic solvent ethanol due to the differnt solvation interaction of both solvents.

Molecular modeling on the inclusion complex between triflumizole and β-cyclodextrin

Force field calculations on the association complex were performed in order to obtain some information about the geometry of the host–guest complex and which residue of triflumizole is mainly affected by the surrounding molecule. Starting from the crystallographic geometry of β-cyclodextrin, various minimized structures of triflumizole association complexes were calculated. Figure 4 gives two minimized conformations of the complex with various orientations of the guest molecule in the interior of cyclodextrin.

Both conformations are of comparable energy. Evidently, due to the flexibility of the cyclodextrin moeity and the conformation variability of triflumizole, a large number of local conformational minima are possible. Nevertheless the two pictures show that the guest molecule fits quite well into the cavity of the cyclodextrin (A), or some residues of the molecule are located in the interior of the host molecule (B). In both cases parts of the molecule still remain at the surface of the association complex with the possibility of the interaction with solvent molecules.

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