Available online at www.sciencedirect.com
SCIENCE DIRECT®

International Journal of Pharmaceutics 256 (2003) 85–94

international journal of pharmaceutics
www.elsevier.com/locate/ijpharm

Solubility enhancement of low soluble biologically active compounds—temperature and cosolvent dependent inclusion complexation

Helmut Viernstein [a,*], Petra Weiss-Greiler [b], Peter Wolschann [b]

[a] *Institute of Pharmaceutical Technology and Biopharmaceutics, University of Vienna, Althanstrasse 14, A-1090 Vienna, Austria*
[b] *Institute of Theoretical Chemistry and Structural Biology, University of Vienna, Währinger Strasse 17, A-1090 Vienna, Austria*

Received 10 July 2002; received in revised form 27 December 2002; accepted 30 December 2002

Abstract

The solubility enhancement of biologically active compounds was analysed in dependence on temperature, amount of cosolvents and on β-cyclodextrin as complexing agent. The analysis was performed for the systemic fungicide triflumizole (TF), a poorly water soluble compound. All parameters lead to an improvement of the solubility, the largest effect was obtained for host–guest complexation with β-cyclodextrin. Generally, the combination of cosolvents and β-cyclodextrin does not increase the solubility of the compound, because cosolvents destabilize the inclusion complex. At higher cosolvent concentrations the solubility of TF is mainly determined by the solubility of the free non-complexed compound in the solvent mixtures. Raising temperature enhances the solubility in any cases. It could be shown that the temperature dependence of the solubility of TF is controlled by the solubility in the solvent mixtures and not by the inclusion reaction.

Keywords: Solubility; Solubility enhancement; β-Cyclodextrin; Host–guest interaction; Triflumizole

1. Introduction

The solubility of biologically active compounds is often a limiting factor for their applicability. Rodidents, fungicides, insecticides and plant protections substances are mainly organic compounds and many of them show a rather low solubility in water. Therefore, the solubility enhancement of these compounds is an important task in pharmaceutical technology, because it leads to a better bioavailability and to more efficient application, connected with a diminished environmental stress. A broad variety of solubilization methods has been developed (Li et al., 1999; Granero et al., 1999; Buvari-Barcza and Barcza, 2000; Jinno et al., 2000; Mura et al., 2001), reaching from changes of the physicochemical parameters of the solution, including pH adjustment and temperature variation, up to the application of cosolvents and excipients, like complexing agents or surfactants.

The physicochemical reason for a low solubility of apolar compounds in water is the competition between selfassociation and solvation. Several approaches have been used to explain the solubility of organic compounds as well as its temperature dependence (Pose-Vilnarnovo et al., 2001). ΔH values of the dissolubility can be measured directly from the temperature dependence of the saturation concentration, whereas for the free energy (ΔG) approxima-

---

[*] Corresponding author. Tel.: +43-1-4277-55402; fax: +43-1-4277-9554.
E-mail address: helmut.viernstein@univie.ac.at (H. Viernstein).

0378-5173/03/$ – see front matter © 2003 Elsevier Science B.V. All rights reserved.
doi:10.1016/S0378-5173(03)00065-6

tions have to be applied (Reinwald and Zimmermann, 1998).

Prediction models for the solubility of some organic compounds give insight which physicochemical parameters could be responsible for the solubility in aqueous solution. As experimental parameters the partition coefficients between octanol and water and the melting point have been suggested (Ran et al., 2001). Theoretically based parameters were molecular connectivity and shape descriptors, as well as electrotopological indices (McElroy and Jurs, 2001; Huuskonen, 2000; Klopmann et al., 1992; Kühne et al., 1995).

The solubility enhancement of poorly soluble compounds can be induced by changes of temperature, solvation properties using different cosolvent compositions, and inclusion reactions with macrocyclic compounds like cyclodextrins. A formulation of triflumizole (TF), a systemic fungicide, with β-cyclodextrin (β-CD) has been already proven to be more efficient than a saturated solution of the compound in water or the standard formulation as a suspension concentrate (Klein et al., 1995).

In the present study, the solubility enhancement of TF was investigated. A comparison of the different methods of solubility improvement, in particular investigations on the inclusion reaction in different aqueous solvent mixtures was performed.

2. Materials and methods

2.1. Materials

Triflumizole (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 No. 99 387-89-0, has curative and protective action against *Gymnosporangium* and *Venturia* spp. in pome fruit, against powdery Erysiphaceae in fruits and vegetables, against *Fusarium*, *Fulvia* and *Monilinia* spp. as well as *Helmintosporium*, *Tilletia* and *Ustilago* spp. in cereals. The compound was provided by Nippon Soda Co. Ltd. (Japan) with a purity of >99%. β-CD was obtained from Roquette Frères (Lestrem, France) as Kleptose® with a humidity of 14% (w/w). Ethanol, 2-propanol, 2-butanol and 1,4-dioxane were of analytical reagent grade; the water used in this study was bidistilled.

2.2. Solubility studies

Solubility measurements and the determination of the saturation concentrations were carried out adding excess amounts of TF to water, water/cosolvent mixtures and β-CD-solutions, respectively. After stirring the samples in a temperature controlled water bath until equilibrium was reached (generally 24 h, and 36 h for the complexation with β-CD), 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 295 nm. The saturation concentrations were estimated at different temperatures (25 °C, 30 °C, 34 °C, 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 different cosolvents (ethanol, 2-propanol, 2-butanol and 1,4-dioxane) were prepared at concentrations of 4, 8, and 12% (w/w).

Stock solutions of β-CD 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 complexation constants were estimated by the solubility method, assuming an one-step equilibrium, according to the method of Higuchi and Connors (Higuchi and Connors, 1965).

3. Results and discussion

3.1. Solubility of TF in various mixtures with cosolvents

The saturation concentration of TF in pure water was estimated as 4.48×10^{-5} mol/l (at 25 °C). The dependence of the solubility in water on the addition of some protic (ethanol, 2-propanol, 2-butanol) and one aprotic (1,4-dioxane) cosolvents is depicted in Fig. 1.

Increasing amounts of cosolvent enhance the solubility of TF, caused by stronger solvation affinity

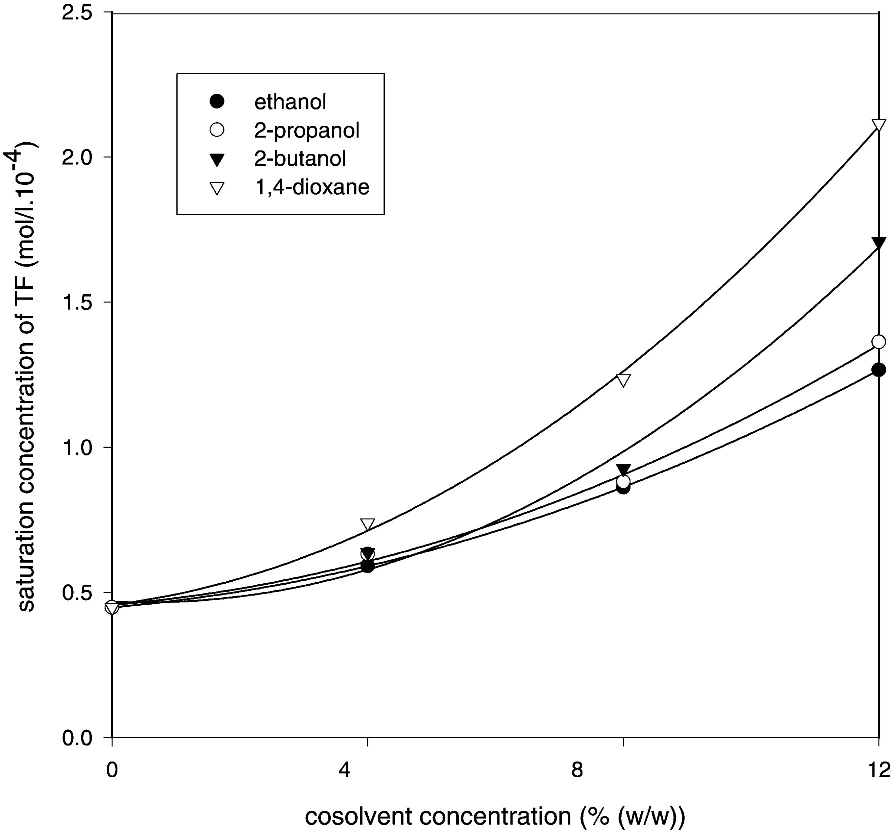

Fig. 1. Solubility of TF in aqueous solution in dependence on the concentration of organic cosolvents (ethanol, 2-propanol, 2-butanol, 1,4-dioxane; saturation concentrations in mol/l, cosolvent concentration in % (w/w)).

of these less polar molecules. The concentrations of different cosolvents given on the axes of abscissa of the diagram are not comparable on molar basis. But even drawings on the saturation concentrations of TF against cosolvent concentrations in mol/l or against various solvent parameters do not lead to a common correlation. This is an indication that specific interactions with the individual solvent molecules predominate in comparison to the influence of general physico-chemical parameters like the dielectric constant or others. The solubility enhancement of the alcohols is more similar compared to the aprotic 1,4-dioxane, which increases the solubility of TF slightly stronger. Nevertheless, the polarity of the solvating molecules seems to be one parameter, which influences the solvation, as for alcohols 2-butanol shows a more pronounced saturations concentration dependence.

3.2. Temperature dependence of the solubility of TF

Measurements at different temperatures have shown that an increase of the temperature leads to a solubility enhancement of TF. To obtain some information about the thermodynamics of the solubilization process the temperature dependence of the saturation concentration of TF in water and in cosolvent mixtures was determined in order to calculate the molar enthalpy of solution (ΔH). According to the Van't Hoff's equation for the temperature dependence of the saturation concentration (cs)

$$\ln(\mathrm{cs}) = \left(\frac{-\Delta H}{R}\right)\left(\frac{1}{T}\right) + \mathrm{const.}$$

where the logarithm of the saturation concentration is correlated with a temperature dependent term and a constant, the slope of the linear correlation line

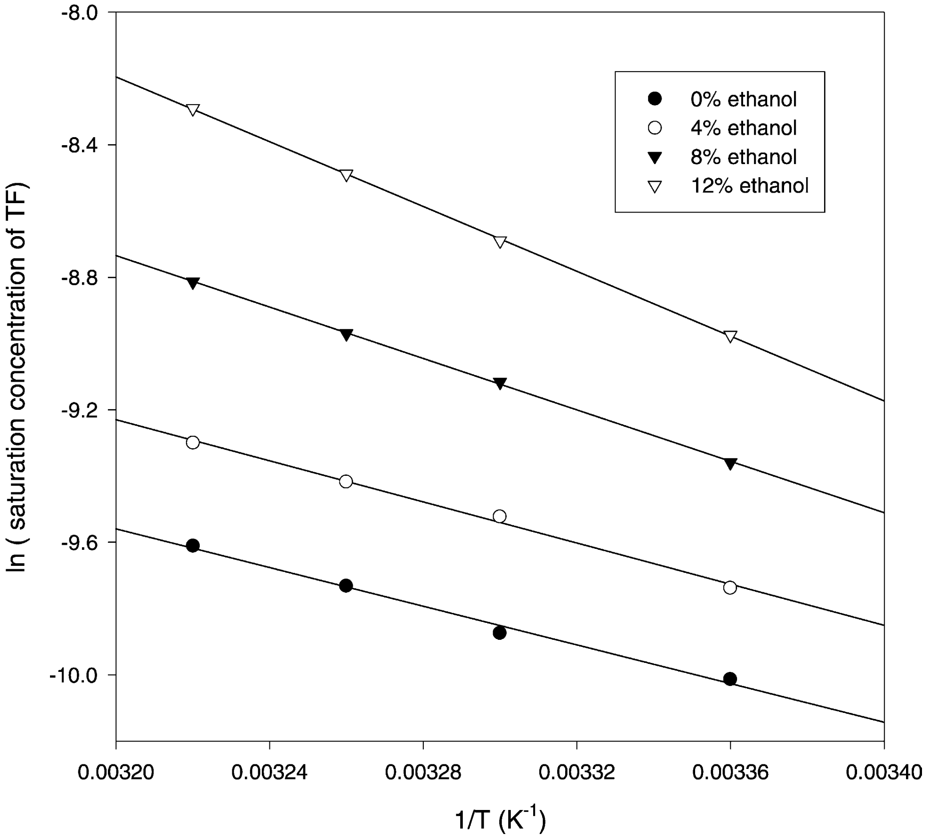

Fig. 2. Van't Hoff's plot of the logarithms of the saturation concentration of TF against 1/T for water (0% (w/w) and various concentrations of ethanol in aqueous solution.

allows to estimate the ΔH values (R equals to the gas constant, T the absolute temperature). A plot of the saturation concentrations against $1/T$ for different concentrations of ethanol in water is shown in Fig. 2.

The determined enthalpies for water and cosolvent mixtures at various concentrations are given in Table 1.

Generally, the enthalpies of solution increase with higher concentrations of cosolvents, as a consequence of the stronger affinity between cosolvent molecules and the solute. Caused by the large experimental error in the estimation of the enthalpies, no clear tendency can be recognized for different cosolvents, but there seems to exist also an increase of the enthalpy values for less polar cosolvents.

Table 1
Enthalpies of solution of TF in various solvent mixtures (ΔH in kJ/mol, the accuracy is ±4 kJ/mol)

Conc. (% (w/w))	Ethanol	2-Propanol	2-Butanol	1,4-Dioxane
4	26	38	30	35
8	32	34	53	39
12	40	41	55	44

The enthalpy of solution for pure water is $\Delta H = 24.0 \pm 1$ kJ/mol.

3.3. Solubility enhancement of TF by inclusion complexation with β-CD

The encapsulation of TF by cyclodextrins leads to a pronounced solubility enhancement of the compound (Viernstein et al., 1994; Klein et al., 1995, 2000b). The concentration of dissolved TF as a function of the β-CD concentration in water is depicted in Fig. 3.

The solubility of TF is increased by the host–guest interaction with β-CD by a factor of 3.75 at a β-CD concentration of $c = 6 \times 10^{-2}$ mol/l at 25 °C. The

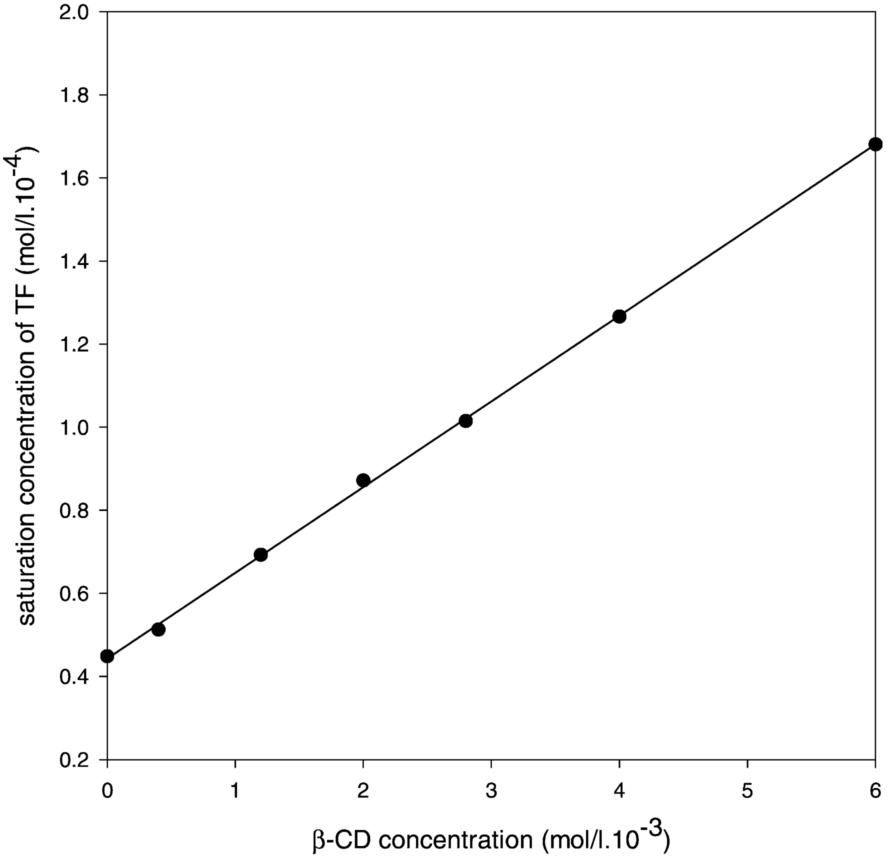

Fig. 3. Concentration of dissolved TF at various concentrations of β-CD.

solubility enhancement of the complexation with β-CD is much higher than caused by the addition of cosolvents, for example, the increase of the solubility of TF in 12% (w/w) ethanol cosolvent mixture results only in a factor of 2.8 (12% (w/w) 2-propanol:3.0; 12% (w/w) 2-butanol:3.8; 12% (w/w) (1.5 mol/l) 1,2-dioxane:4.7). The higher saturation concentration of TF (dissolved TF, consisting of free and complexed compounds) with β-CD is the consequence of the interaction of the less polar TF with the hydrophobic interior of the host. Former investigations on the geometry of TF-β–CD complexes have shown that only one substituent of TF is located in the hosts cavity, whereas the residual part of the molecule is associated at the external molecular surface of β-CD (Klein et al., 1995; Viernstein et al., 1994). There are small energy differences for the inclusion of the three side chains of TF. Evaluation of prediction models of organic compounds with various cyclodextrins allow the calculation of the free energies, but moreover, lead also to some molecular parameters, which are important for the complexation reaction. It could be shown that for many molecules the association at the external surface of the host molecule is essential for the complexation affinity, in particular for complexes with the smaller host, α-CD (Klein et al., 2000a,b). Quantitatively the complexation of TF with β-CD is expressed by the overall association constant K ($K = 476$ l/mol for 25 °C) (Klein et al., 1995).

3.4. Temperature dependence of the inclusion complexation of TF with β-CD

The equilibrium constant of the inclusion reaction decreases at higher temperatures, according to a negative ΔH value of the overall reaction. A plot of the

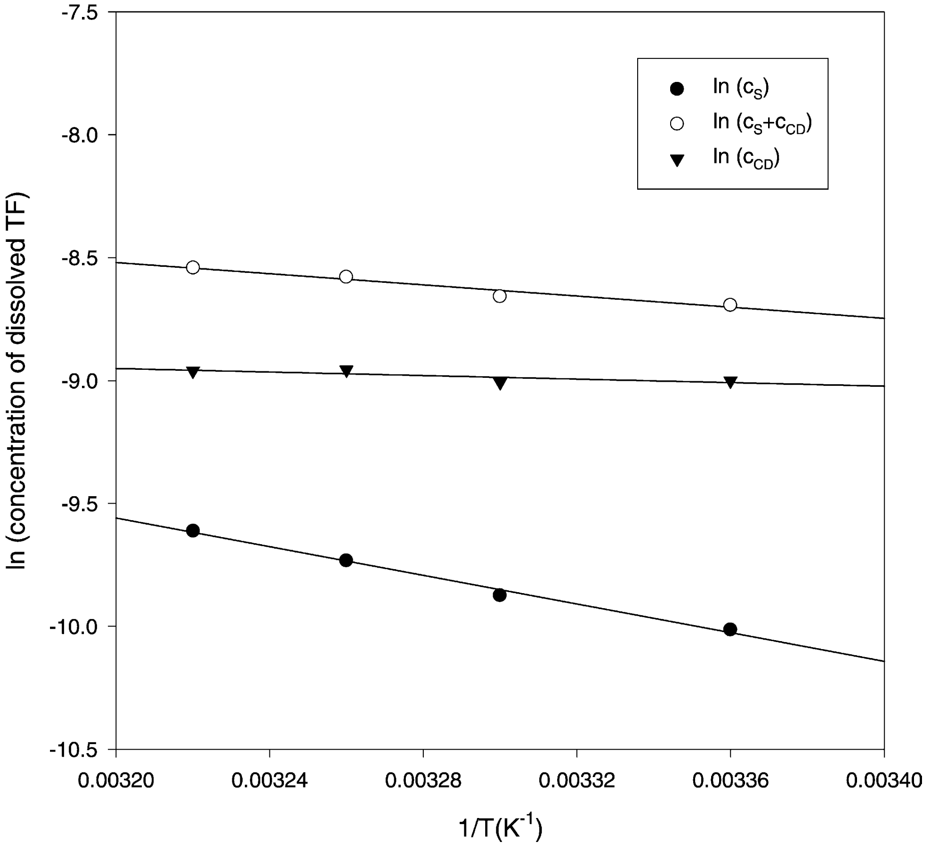

Fig. 4. Van't Hoff's plot of the logarithm of the saturation concentration of TF (cs) in water against 1/*T* compared to the temperature dependence of the experimentally observed concentration of TF in the presence of β-CD ($c = 6 \times 10^{-3}$ mol/l) (cs + cCD) and of the calculated concentration of complexed TF (cCD).

temperature dependence of the saturation concentration of TF in water (cs) compared to the concentration of complexed TF (cCD) and of the experimentally determined concentration of the sum of TF associated in the complex and dissolved free compound (cs + cCD) is given in Fig. 4.

The temperature dependences of the saturations concentration (cs) and of the experimentally observed amounts of dissolved TF with β-CD show a similar behaviour. The temperature dependence of the calculated concentration of the complexed TF (cCD) appears to be very small and results in a ΔH value of about 3.1 ± 2 kJ/mol, as the sum between the overall reaction enthalpy and the enthalpy of solution. This is a strong indication that the temperature dependence of the saturation concentration of TF in the presence of β-CD contributes predominantly to the overall reaction enthalpy. A detailed thermodynamic analysis of the system will be given elsewhere (Viernstein et al., in preparation).

3.5. *Influence of cosolvents on the inclusion complexation with β-CD*

The combined effect of cosolvency and CD inclusion complexation is interesting as it is a priori not clear, if there exist a synergistic or an antagonistic effect. Both phenomena were found for various systems (Zung et al., 1991; Pitha and Hoshino, 1992; Connors et al., 1992; Reer and Müller, 1993; Furuta et al., 1993), and the dependences of the solubility were described and explained by different models (Li et al., 1999). In the case of TF a diminished solubility at small amounts of cosolvent can be observed. The

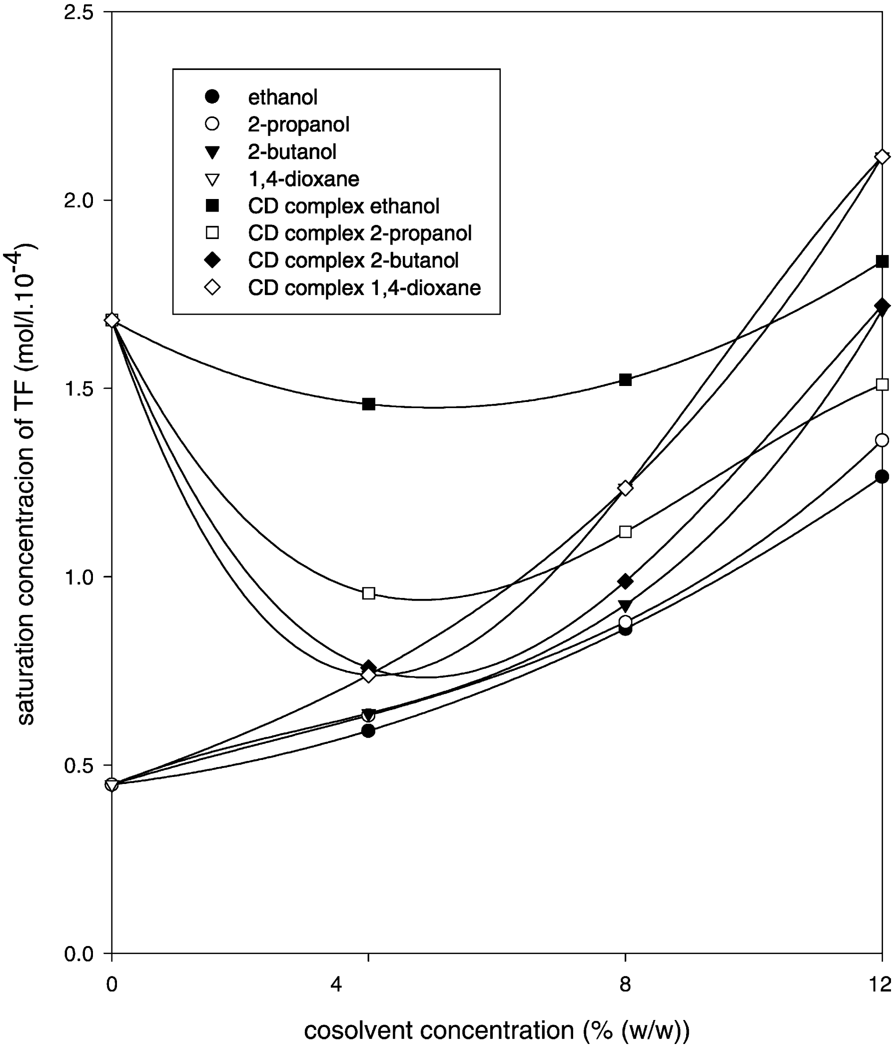

Fig. 5. Solubility of TF in water and in cosolvent mixtures in dependence on the concentration of cosolvents (ethanol, 2-propanol, 2-butanol, 1,4-dioxane) (saturation concentrations in mol/l, cosolvent concentration in % (w/w)). Saturation concentrations are given and compared to the corresponding ternary systems. The concentration of β-CD is equal to 6×10^{-3} mol/l.

dependence of the solubilities on the cosolvent concentration, together with the solubility of TF without addition of β-CD is demonstrated in Fig. 5.

The concentration of dissolved TF decreases upon addition of cosolvent (4% (w/w)) and increases then for higher amounts of cosolvents (c >8% w/w). The solubility dependence curve is a superimposition of the saturation concentration dependence of solvent mixtures without β-CD and the destabilization of the TF–β-CD inclusion complex in solvent mixtures of slightly lower polarity. The reason for this destabilization is the competition of the cosolvent molecules as guests with TF molecules in the interior of the host molecules. Evidently, this competition is stronger for the more apolar and more bulky alcohols. After the complete decomposition of the host–guest complexes, the solubility is controlled by the solubility of TF in the solvent mixture without β-CD. To obtain some

Table 2
Equilibrium constants of TF–β-CD complexes (in l/mol at 25 °C) in dependence on cosolvent concentrations

Conc. (% (w/w))	Ethanol	2-Propanol	2-Butanol	1,4-Dioxane
4	242.6	88.1	33.7	40.4
8	127.9	45.7	11.5	18.7
12	73.7	18.2	0.9	10.6

quantitative information about the destabilization the overall equilibrium constants in water and in various solvent mixtures were estimated. The results are summarized in Table 2.

The strong destabilizing influence of the cosolvents can be clearly seen. The equilibrium constants decrease with increasing concentrations of the respective alcohol. 2-Propanol and 2-butanol cause a much stronger destabilization. In the case of 1,4-dioxane the same trend can be observed, but the equilibrium constants are slightly higher, than it would be expected from the polarity of the solvent. Some approach for this pronounced solvent dependency on the equilibrium constant was already performed using a two-step competition model (Klein et al., 1995).

3.6. *Influence of the temperature on the inclusion complexation with β-CD in the presence of cosolvents*

Van't Hoff's plots of the TF concentrations according to Fig. 4 for the ternary system including β-CD ($c = 6 \times 10^{-3}$ mol/l) and ethanol (4% (w/w)) are shown in Fig. 6. A similar picture as in Fig. 4 is

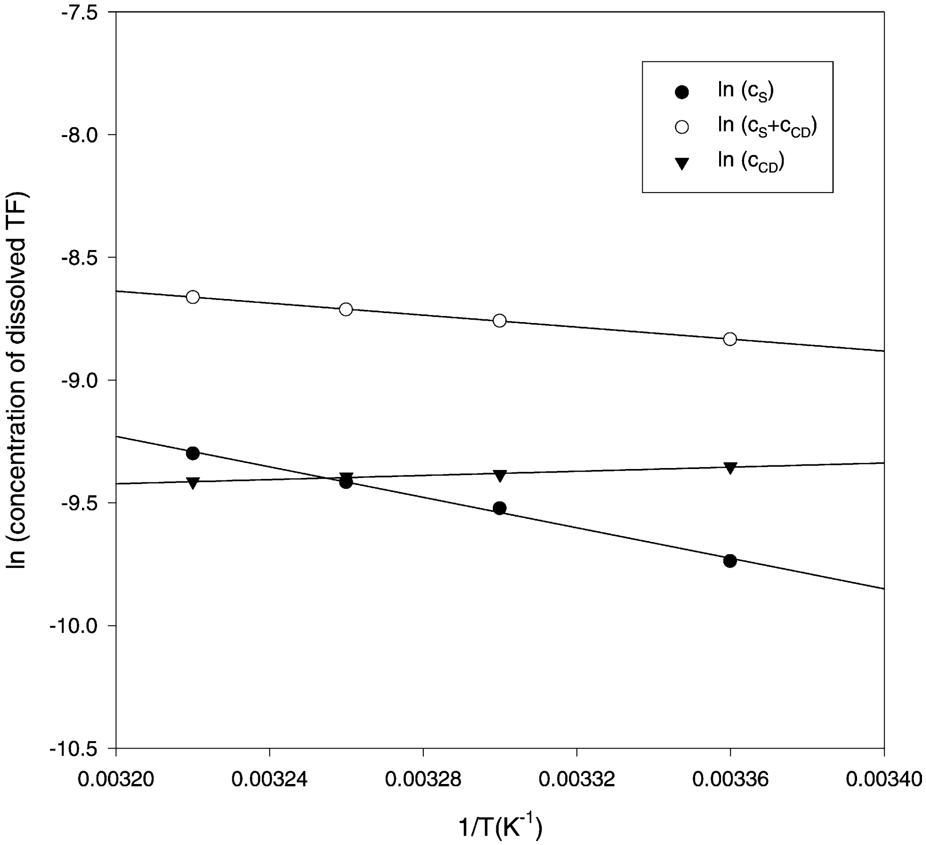

Fig. 6. Van't Hoff's plot of the logarithm of the saturation concentration of TF (cs) in 4% (w/w) ethanol against 1/*T* compared to the temperature dependence of the experimentally observed concentration of TF in the presence of β-CD ($c = 6 \times 10^{-3}$ mol/l) (cs + cCD) and of the calculated concentration of complexed TF (cCD).

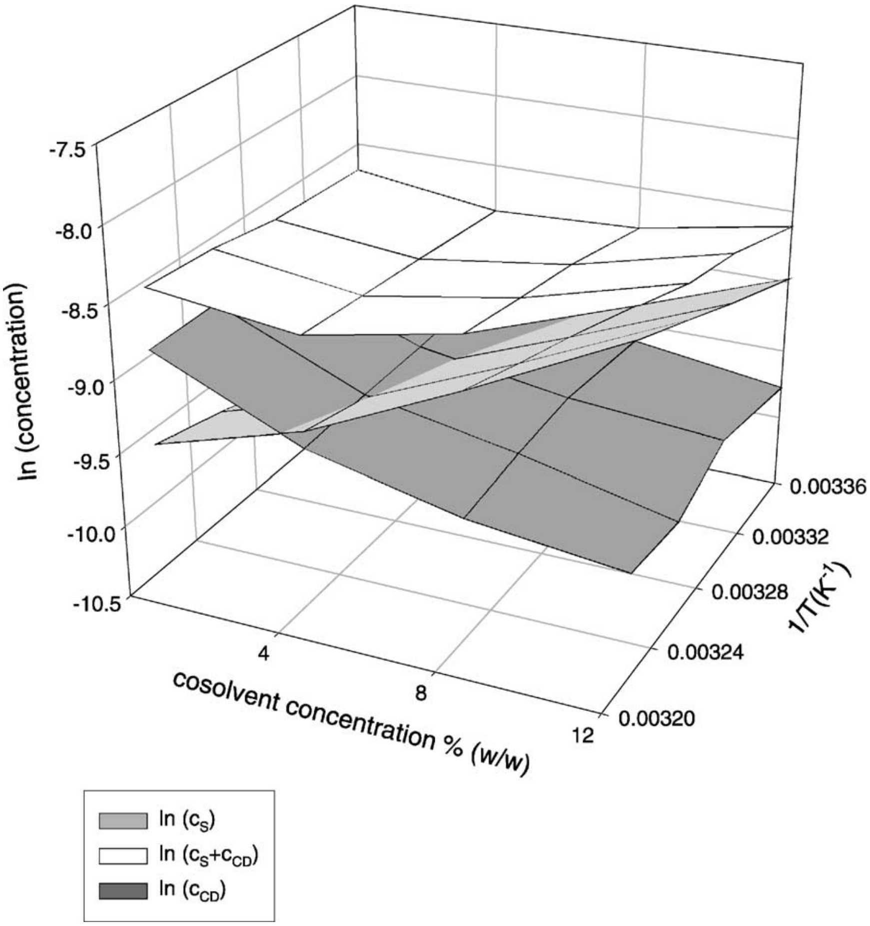

Fig. 7. 3D plots of the Van't Hoff's correlations of the concentrations of TF for different concentrations of ethanol as cosolvent. The plane drawn in white corresponds to the experimentally determined concentration of TF (free and complexed), β-CD ($c = 6 \times 10^{-3}$ mol/l). The light grey plane represents the dependence of the saturation concentration of TF without β-CD and the dark grey surface is calculated as difference between the experimentally determined concentration of TF and the saturations concentrations in the respective cosolvents.

obtained, but the concentration of complexed TF (cCD) is significantly smaller. Again the slope for the correlation of ln(cCD) against $1/T$ is close to zero or even slightly positive.

Generally, the solubility of TF as guest molecule in a β-CD clathrate in pure water increases with raising temperature, although the stability of the complex decreases. Higher temperatures increase also the solubility of TF at any cosolvent concentration.

According to Fig. 2 and Fig. 4 the dependence of the logarithm of the concentrations against $1/T$ are shown for ethanol in a 3D plot (Fig. 7).

In analogy to the situation in pure water the saturation concentration of TF without β-CD is strongly temperature dependent for all ethanol concentrations, and, remarkably, the temperature dependence increases at higher alcohol concentrations. At these higher cosolvent concentrations the surfaces describing the overall concentration of TF and the solubility in pure solvent mixtures approach each other, as the concentration of the complexed compound (cCD) decreases. The concentration cCD is more or less independent on the temperature for all cosolvent concentrations. Similar diagrams have been obtained for the other cosolvents.

4. Conclusion

The solubility of TF increases with raising temperatures, by the addition of cosolvents and to the highest extent by inclusion complexation with β-CD.

The increase of the solubility of TF in pure water can be given by the factor 1.5 by changing the temperature from 25 to 38 °C. A solubility enhancement by a factor of 3.75 is obtained for the inclusion complexation with β-CD.

Inclusion complexation together with the use of cosolvents does not, generally, improve the solubility as the destabilization of the association complex by the cosolvent molecules occurs even at low amounts of cosolvents. At higher cosolvent concentrations no inclusion complexes and, therefore, no contribution of this reaction to the solubility can be observed, except for the more polar cosolvent ethanol, where the solubility at higher ethanol concentrations is indeed higher with CD as in the cosolvent mixture alone.

The temperature dependence of the concentration of TF in the presence of β-CD is mainly controlled by the temperature dependence of the solubility in the solvent mixtures without the inclusion agent.

Acknowledgements

The authors would like to thank Mrs. E. Liedl for technical assistance and the Fonds zur Förderung der wissenschaftlichen Forschung (FWF, Grant No. P 15431) for financial support.

References

Buvari-Barcza, A., Barcza, L., 2000. Changes in the solubility of β-cyclodextrin on complex formation: guest enforced solubility of β-cyclodextrin inclusion complexes. J. Incl. Phenom. 36, 355–370.

Connors, K.A., Mulski, M.J., Paulson, A., 1992. Solvent effects on chemical processes. 2. Binding constants of methyl orange with α-cyclodextrin in binary aqueous-organic solvents. J. Org. Chem. 57, 1794–1798.

Furuta, T., Yoshii, H., Miyamoto, A., Yasunishi, A., Hirano, H., 1993. Effects of water and alcohol on the formation of inclusion complexes of D-limonene and cyclodextrins. Supramol. Chem. 1, 321–325.

Granero, G., de Bertorello, M.M., Brinon, M.C., 1999. Solubility profiles of some isoxazolyl-naphthoqinone derivatives.

Higuchi, T., Connors, K.A., 1965. Phase-solubility techniques. Adv. Anal. Chem. Instr. 7, 117–212.

Huuskonen, J., 2000. Estimation of aqueous solubility for a diverse set of organic compounds based on molecular topology. J. Chem. Inf. Comput. Sci. 40, 773–777.

Jinno, J., Oh, D.M., Crison, J.R., Amidon, G.L., 2000. Dissolution of ionisable water-insoluble drugs: the combined effect of pH and surfactant. J. Pharm. Sci. 89, 268–274.

Klein, Ch.Th., Köhler, G., Mayer, B., Mraz, K., Reiter, S., Viernstein, H., Wolschann, P., 1995. Solubility and molecular modelling of triflumizol–β-cyclodextrin inclusion complexes. J. Incl. Phenom. 22, 15–32.

Klein, C.T., Polheim, D., Viernstein, H., Wolschann, P., 2000a. Prediciting the free energies of complexation between cyclodextrins and guest moleucles: linear versus nonlinear models. Pharm. Res. 17, 358–365.

Klein, Ch.Th., Viernstein, H., Wolschann, P., 2000b. Inclusion complexation with cyclodextrins. Minerva Biotech. 12, 287–292.

Klopmann, G., Wang, S., Balthasar, D.M., 1992. Estimation of aqueous solubility of organic molecules by the group contribution approach. Application to the study of biodegradation. J. Chem. Inf. Comput. Sci. 32, 447–482.

Kühne, R., Ebert, R.-U., Kleint, F., Schmidt, G., Schüürmann, G., 1995. Group contribution methods to estimate water solubility of organic chemicals. Chemosphere 30, 2061–2077.

Li, P., Zhao, L., Yalkowsky, S.H., 1999. Combined effect of cosolvents and cyclodextrin on solubilization of nonpolar drugs. J. Pharm. Sci. 88, 1107–1111.

McElroy, N.R., Jurs, P.C., 2001. Prediction of aqueous solubility of heteroatom containing organic compounds from molecular structure. J. Chem. Inf. Comput. Sci. 41, 1237–1247.

Mura, P., Faucci, M.T., Manderioli, A., Bramanti, G., 2001. Multicomponent systems of Econazole with hydroxy acids and cyclodextrins. J. Incl. Phenom. 39, 131–138.

Pitha, J., Hoshino, T., 1992. Effect of ethanol on formation of inclusion complexes of hydroxypropylcyclodextrins with testosterone or with methyl orange. Int. J. Pharm. 80, 243–251.

Pose-Vilnarnovo, B., Perdomo-Lopez, I., Echezarreta-Lopez, M., Schroth-Pardo, P., Estrada, E., Torres-Labandeira, J.J., 2001. Improvment of water solubilità of sulfamethizole through its complexation with β- and hydroxypropyl-β-cyclodextrin. Characterization of the interaction in solution and in solid state. Eur. J. Pharm. Sci. 13, 325–331.

Ran, Y., Jain, N., Yalkowsky, S.H., 2001. Prediction of aqueous solubility of organic compounds by the general solubility equation (GSE). J. Chem. Inf. Comput. Sci. 41, 1208–1217.

Reer, O., Müller, B.W., 1993. Investigation of the influence of cosolvents and surfactants on the complexation of dexamethasone with hydroxypropyl-β-cyclodextrin by use of a simplex lattice design. Eur. J. Pharm. Biopharm. 39, 105–111.

Reinwald, G., Zimmermann, I., 1998. A combined calorimetric and semiempirical quantum chemical approach to describe the solution thermodynamics of drugs. J. Pharm. Sci. 87, 745–750.

Viernstein, H., Reiter, S., Wolschann, P., 1994. Solubility enhancement of triflumizole by host–guest interaction with β-cyclodextrin. Monatsh. Chem. 125, 681–689.

Zung, J.B., Pena, A.M., Ndou, T.T., Warner, I.M., 1991. Influence of alcohol addition on the γ-cyclodextrin-pyrene complex. J. Phys. Chem. 95, 6701–6709.