CHEMICAL ENGINEERING TRANSACTIONS Volume 17, 2009
Editor Sauro Pierucci
 ISBN 978-88-95608-01-3 ISSN 1974-9791
DOI: 10.3303/CET0917181

Experimental study of the inclusion of triclosan in hydroxypropyl-β-cyclodextrins

Roberto Lavecchia and Antonio Zuorro
Department of Chemical Engineering, Materials & Environment – Sapienza University
Via Eudossiana, 18 – 00184 Roma (Italy)

Triclosan, an antimicrobial agent with an extremely low water solubility, was included in the hydrophobic cavity of hydroxypropyl-β-cyclodextrins. Complex formation was evaluated by the Higuchi-Connors phase-solubility method and by UV difference spectrophotometry. The results obtained by both methods suggest that an inclusion complex of 1:1 stoichiometry is formed. The estimated association constant was close to 500 M^{-1}, indicating a considerably high stability. As a result of complexation, triclosan solubility in aqueous ethanol (10% v/v) showed an almost 70-fold increase.

1. Introduction

Triclosan [5-chloro-2-(2,4-dichlorophenoxy)phenol] is an antimicrobial agent commonly used in consumer and medical products such as mouthwashes, toothpastes, surgical scrubs, and more recently suture materials, for its ability to inhibit the growth of a wide range of microorganisms (Jones et al., 2000; Edmiston et al., 2004). Its mechanism of action is thought to involve inhibition of enoyl-acyl carrier protein reductase, a highly conserved enzyme in bacteria that is responsible for fatty acid biosynthesis (McMurry et al., 1998; Heath et al., 2000; Schweizer, 2001). In addition to biocidal activity, triclosan has been found to exhibit remarkable anti-inflammatory properties (Skaare et al., 1997; Mustafa et al., 1998).
Chemically, triclosan is a chlorinated diphenyl ether with some structural similarities to phenols (Figure 1). Due to its hydrophobic character, triclosan has an extremely low water solubility (about 10 μg/mL at 25 °C) which limits its applicability in the pharmaceutical and materials industries.
To overcome the above limitations we have explored the possibility of incorporating this compound into modified cyclodextrins. Cyclodextrins are cyclic oligosaccharides with a hydrophilic outer surface and a hydrophobic cavity in the center (Del Valle, 2004; Brewster and Loftsson, 2007). If a lipophilic guest molecule has the appropriate size and shape it can be hosted in the cavity, with a significant increase in its aqueous solubility.
In this study we have focused our attention on the inclusion of triclosan in hydroxypropyl-β-cyclodextrin, a toroidal molecule with a cavity size suitable for hosting the triclosan molecule. As a first step to understanding the character and properties of the association, we evaluated the stoichiometry and stability of the complex. The effects on triclosan solubility were also investigated.

Please cite this article as: Lavecchia R. and Zuorro A., (2009), Experimental study of the inclusion of triclosan in hydroxypropyl-beta-cyclodextrins, Chemical Engineering Transactions, 17, 1083-1088 DOI: 10.3303/CET0917181

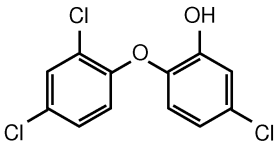

Figure 1 – Molecular structure of triclosan.

2. Experimental

2.1 Materials

Triclosan (Irgasan® DP-300), with a purity greater than 99%, was obtained from Ciba Spa (Origgio, Italy). 2-hydroxypropyl-β-cyclodextrins (Cavitron 82006), with a substitution degree of 6.1 and a purity of 82.5%, were purchased from Cerestar Italia Spa (Castelmassa, Italy). Ethanol, with a purity greater than 99.5%, was from Carlo Erba (Milano, Italy). All chemicals were used as received and aqueous solutions were made up in bidistilled water.

2.2 Methods

Phase-solubility studies

The effects of 2-hydroxypropyl-β-cyclodextrins (HPβCD) on the solubility of triclosan were determined in aqueous ethanol (10% v/v) containing HPβCD at varying concentrations (0–120 mM). An excess amount of triclosan was added to each medium in magnetically stirred flasks and the suspensions formed heated at 60 °C for 60 min. After equilibration at room temperature (18–20 °C) for further 48 h, aliquots of the suspensions were passed through a 0.45 μm nylon membrane filter and the filtrates analyzed spectrophotometrically. Measurements were made at 282 nm, where the absorption spectrum of triclosan has a sharp peak (Figure 2).

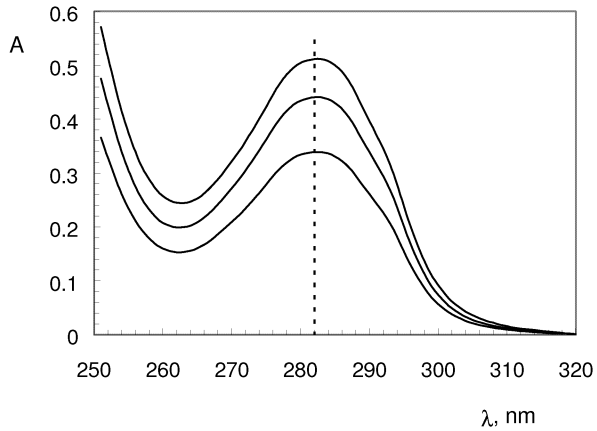

Figure 2 – UV absorption spectra of triclosan in aqueous ethanol (10% v/v) at different triclosan concentrations.

UV spectrophotometry studies

UV difference spectrophotometry was used to characterize the association between triclosan and HPβCD. Experiments were performed by a double-beam UV-Vis spectrophotometer (Perkin Elmer, Lambda 25) with 1–cm quartz cuvettes.

Aqueous solutions (30% ethanol in water) were prepared by adding increasing amounts of HPβCD (from 0.12 and 84.9 mM) to 0.46 mM triclosan. Absorption spectra of these solutions were recorded against a reference solution containing triclosan (at the same concentration) in aqueous ethanol. Measurements were made at room temperature over the wavelength range 250–320 nm. An example of the resulting spectral curves is given in Figure 3.

3. Results and Discussion

Figure 4 shows the phase-solubility diagram of triclosan in aqueous HPβCD solutions. As can be seen, the solubility of triclosan in water is greatly enhanced by complexation with HPβCD. At the highest cyclodextrin concentration (120.4 mM) the solubility undergoes an almost 70-fold increase (from 0.19 to 13.5 mM) with respect to the value observed in the absence of cyclodextrins.

Examination of the shape the phase-solubility diagram reveals an A_L-type behavior (Del Valle, 2004), i.e., a linear increase of solubility as a function of cyclodextrin concentration. To evaluate the stoichiometry and stability constant for the host (H) – guest (G) complex:

$$mH - nG \Rightarrow H_m G_n$$

we correlated the solubility data by several equilibrium complexation models.

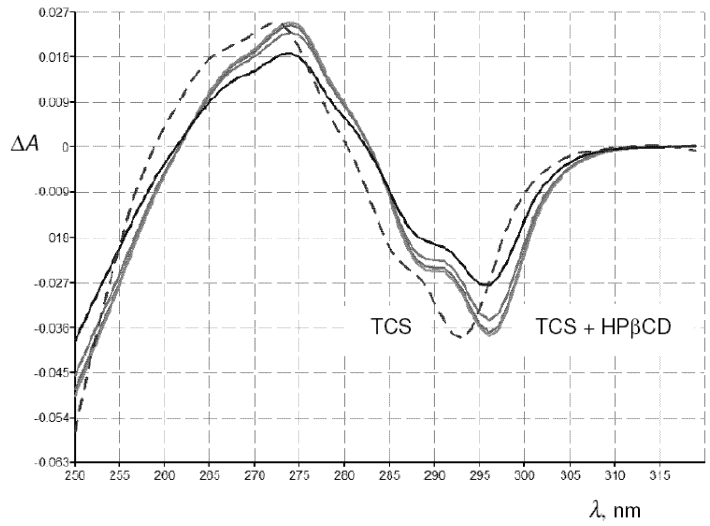

*Figure 3 – Difference absorption spectra of triclosan (TCS) alone and in the presence of varying amounts of HP*βCD.

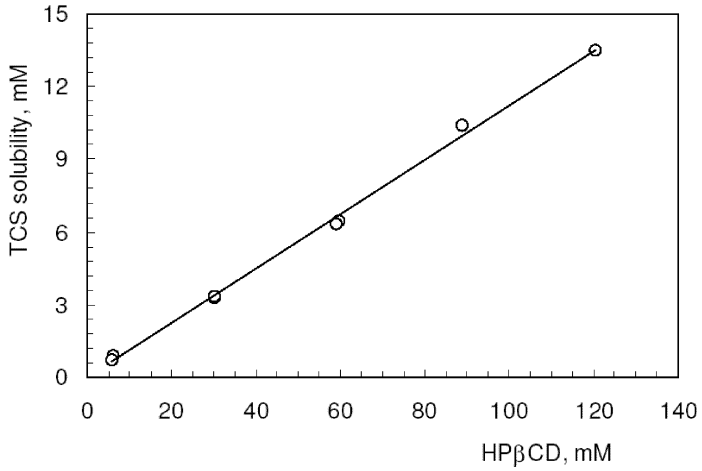

Figure 4 – Phase-solubility plot of triclosan in aqueous HPβCD solutions.

The best result was obtained for $m = n = 1$, this suggesting the formation of a 1:1 complex between triclosan and HPβCD. The associated equilibrium constant:

$$K_{1:1} = \frac{[HG]}{[H][G]} \tag{1}$$

was calculated from the slope (σ) and intercept (ρ) of the straight line in the solubility plot as reported by Higuchi and Connors (1965):

$$K_{1:1} = \frac{\sigma}{\rho(1-\sigma)} \tag{2}$$

obtaining $K_{1:1} = 582.8 \pm 14.8\ M^{-1}$.

To quantify the spectral changes resulting from the addition of HPβCD to triclosan solutions we examined the measured values of ΔA at various wavelengths. As in the case of solubility measurements, we assumed different equilibrium models for complex formation and correlated, with the resulting expressions, $\Delta A/A_0$ as a function of cyclodextrin concentration (Connors, 1997). Absorbances were read at 296 nm because of the higher signal intensity in the difference spectra.

Again, the best fit was obtained for the 1:1 association model:

$$\frac{\Delta A}{A_0} = \frac{aK_{1:1}[H]}{1 + K_{1:1}[H]} \tag{3}$$

which gave $K_{1:1} = 415.2 \pm 81.3\ M^{-1}$. A comparison between experimental and calculated results is shown in Figure 5.

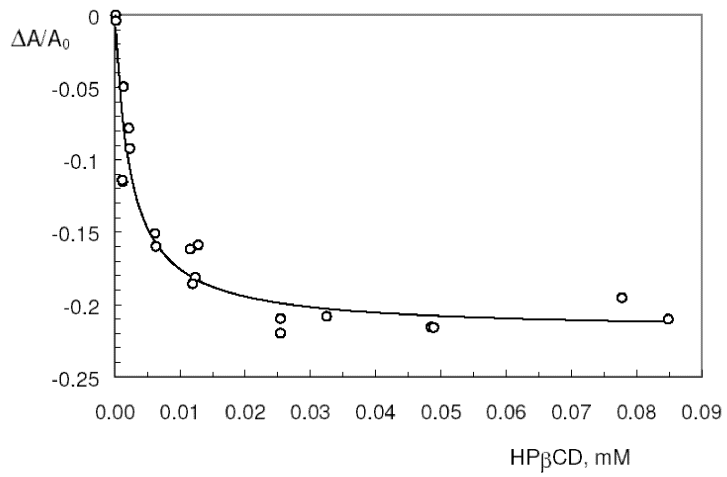

Figure 5 – Correlation of spectrophotometric data by the 1:1 model. A and A_0 are the absorbances at 296 nm of triclosan solutions with or without cyclodextrins.

The results from both solubility and spectrophotometric measurements suggest that the interactions between triclosan and HPβCD lead to the formation of a complex with 1:1 stoichiometry. This stoichiometry is consistent with the molecular dimensions of triclosan and those of the hydrophobic cavity of HPβCD (Qian et al., 2008). The average value of the equilibrium constant estimated by the two methods is around 500 M^{-1}, which indicates a quite high stability for the association complex (Connors, 1987). Overall, the findings from this study strongly support the possibility of using HPβCD to significantly enhance the solubility of triclosan in aqueous systems and enlarge the range of applications of this antimicrobial agent.

4. Acknowledgements

We would like to thank Ciba Spa (Origgio, Italy) and Cerestar Italia Spa (Castelmassa, Italy) for their kind gift of products. We are also grateful to Marco Trosi for his contribution to the experimental part of this work.

5. References

Brewster M.E. and Loftsson T., 2007, Cyclodextrins as pharmaceutical solubilizers, Adv. Drug Deliv. Rev. 59, 645–666.

Connors, K. A., 1987, Binding constants: the measurement of molecular complex stability, John Wiley & Sons: New York.

Connors, K. A., 1997, The stability of cyclodextrin complexes in solution, Chem. Rev. 97, 1325–1358.

Del Valle E.M.M., 2004, Cyclodextrins and their uses: a review, Process Biochem. 39, 1033–1046.

Edmiston C., Schmitt A., Krepel C. and Seabrook G., 2004, Impact of triclosan-impregnated suture on in vitro adherence of nosocomial surgical pathogens, Am. J. Infect. Control 32, E108–E108.

Heath R.J., Li J., Roland G.E. and Rock C.O., 2000, Inhibition of the staphylococcus aureus NADPH-dependent enoyl-acyl carrier protein reductase triclosan and hexachlorophene, J. Biol. Chem. 275, 4654–4659.

Higuchi T. and Connors K.A., 1965, Phase solubility techniques, Adv. Anal. Chem. Instrum. 4, 117–212.

Jones R.D., Jampani H.B. and Lee A.S., 2000, Triclosan: a review of effectiveness and safety in health care settings, Am. J. Infect. Control 28, 184–196.

McMurry L.M., Oethinger M. and Levy S.B., 1998, Triclosan targets lipid synthesis, Nature 394, 531–532.

Mustafa M., Wondimu B., Ibrahim M. and Modéer T., 1998, Effect of triclosan on interleukin-1β production in human gingival fibroblasts challenged with tumor necrosis factor α, Eur. J. Oral Sci. 106, 637–643.

Qian L., Guan Y. and Xiao H., 2008, Preparation and characterization of inclusion complexes of a cationic β-cyclodextrin polymer with butylparaben or triclosan, Int. J. Pharm. 357, 224–251.

Schweizer H.P., 2001, Triclosan: a widely used biocide and its link to antibiotics, FEMS Microbiol. Lett. 202, 1–7.

Skaare A.B., Kjaerheim V., Barkvoll P. and Rolla G., 1997, Does the nature of the solvent affect the anti-inflammatory capacity of triclosan? An experimental study, J. Clin. Periodontol. 24, 124–128.